Toxicological Evaluation of Copper Oxychloride on Carassius Auratus: Determining Lethal Concentrations and Behavioural Responses

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Abstract

Dofs are plant-specific transcription factors involved in diverse functions, including growth, development, hormonal and Abiotic stress response regulation. Although genome-wide analysis of Dof genes has been performed in many species, these genes have yet to be analyzed in lima bean. The current study was aimed at exploring and characterizing the Dof gene family across the genome of the lima bean. Forty PlDof genes in the genome of lima bean were detected during the present study, and named as PlDof1-PlDof40 based on their location on the chromosome in the ascending order. PlDof12 was recorded as the largest gene with 1560 bp CDS, 519 amino acids protein length (PL) and 57.126 kDa protein molecular weight (PMW), while PlDof-23 was found as the smallest one with only 522 CDS, 173 PI and 19.75 kDa PMW, respectively. Nucleus was predicted as a major residence for all 40 PlDof proteins. Isoelectric points and GRAVY were found in the range of 5.35 to 8.91 and -0.269 to -0.746, respectively. The varying number of exons reveals that deletions/additions have occurred during evolution, resulting in variation in motif number. Motif1was conserved in all PlDof genes. The phylogenetic tree reveals that paralogs contributed 33% to the PlDof gene family expansion. Ka/Ks ratios of paralogs reveal that they were all under purifying selection. The promoter region has environmental, developmental, light and hormone-responsive elements that might contribute to the diverse function of PlDof. This study provides a basis for the functional validity of PlDof genes.

Keywords: Lima bean, Dof, genome-wide analysis, evolutionary analysis, transcription factor

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Introduction

Pest is any organism, such as insects, weeds, nematodes, microbes, molluscs, plant pathogens, birds and mammals, which competes with humans for food, destroys their properties or may act as a vector for transmitting different diseases. (Yadav and Devi 2017). Generally, many insects are considered pests, but from an agricultural point of view, any organism, such as a plant or animal, which is out of context is regarded as a pest. (Hill 1987). The most frequent method used to control pests and plant diseases in agricultural regions is through the use of pesticides (Sharifzadeh, Abdollahzadeh et al. 2018).

According to an estimate, 2.5 billion pounds of pesticides are utilized worldwide per year. Pesticides are not only used in agriculture but also used in homes in the form of poisons, sprays and powders to control the harmful organisms such as mosquitoes, rats, fleas, ticks, cockroaches etc. however 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).

Based on the targeted pest organisms, pesticides are classified into various types such as insecticides, fungicides, bactericides, herbicides, acaricides, rodenticides and algaecides, etc (Rajani and Dave 2020). Excessive use of pesticides leads to environmental pollution, including air pollution, soil pollution, water pollution and food contamination, which affect human and non-targeted organisms (Pathak, Verma et al. 2022).

The toxicity of pesticides depends on the number of pesticides used and the time of exposure. Some pesticides are carcinogenic agents for humans and may affect the immune and endocrine systems that causing neuronal disorders, while some pesticides affect fetal development. DDT is an insecticide which is the causal agent of many types of cancers, lung injury, damage to the reproductive organs and congenital disorders, etc (Sharma and Singhvi 2017).

Organophosphate pesticides are very detrimental to vertebrates and may stop the activity of an enzyme called acetylcholinesterase (Mitra, Sarkar et al. 2019). Copper-containing pesticides can cause various kinds of cancer, allergic reactions. Eye 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 it enters the body, can irritate the digestive system (Husak 2015). Pesticides containing copper, like copper sulphate, copper oxychloride, as well as copper carbonate, are sensitizers that can cause allergic reactions, eyelid swelling, digestive system irritation, and harm to the liver, kidneys, and blood cells (Ullah, Khan et al. 2024).

Pesticides act as a major pollutant that affects the aquatic environment and, therefore, can accumulate in the bodies of fish. The accumulation of these harmful pesticides in the tissues and organs of fish can cause serious diseases and mortality in fish. Specific vital organs of fishes, such as liver, gills and kidneys, are affected by pesticides (Sethuraman, Kiros et al. 2013). The exposure of fingerlings of common carp to organophosphate insecticide causes different behavioral 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 hematological changes in fish, such as a decrease in erythrocytes and

hemoglobin, thus leading to anemia in fish (Ismail, Ali et al. 2018). Chlorpyrifos can cause histopathological changes in the kidneys of fish and causes necrosis of the renal tubules (Srivastava, Tiwari et al. 1990). Long-term exposure of fish to Chlorpyrifos can act as a mutagen and induce DNA damage in tissues, specifically higher DNA damage in gill cells (Ali, Nagpure et al. 2009).

The present study was conducted to assess the toxic effects and lethal concentrations of copper oxychloride to *Carassius auratus* at District Swat.

Materials and Methods

Study area

The present study was conducted in the Centre for Animal Sciences and Fisheries, University of Swat, at District Swat, Pakistan.

Sample collection

The *Carasius auratus* were collected from the River Swat using cast nets with the help of local fishermen and were then transported to the laboratory in plastic oxygenated bags, where these fish were kept in aquaria.

Ethical approval

The ethical approval was granted by the ethical committee of the Centre for Animal Sciences and Fisheries, University of Swat.

Acclimatization of Fish

The acclimatization of fish was done in aquaria with a capacity of 41 litres of water for about three weeks. The temperature of the water was maintained at 23±2^{o}C with an average pH of 7.5. The dissolved oxygen level was maintained at 5mg/L with the help of an aerator motor pump. The fish were fed twice based on their body weight with fish pellet feed and rice brans (Ullah, Ullah et al. 2021). 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 the 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 that occurred. The number of dead fish was counted and then removed from the aquaria (Ullah, Khan et al. 2024).

Data analysis

The data were analyzed by using IBM SPSS Version 21 statistical software. A one-way ANOVA test was applied to compare the variables among the control different experimental groups.

Results

The present study was designed to investigate the adverse effects of copper oxychloride (Cobox) on *Carassius auratus*. In this study, the *Carassius auratus* were exposed to various concentrations of copper oxychloride 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 Copper oxychloride on the behaviour of *Carassius auratus*

The fish of the control group or pesticide-free group showed normal behaviour, and no mortality was recorded. The fish of the exposed groups showed different abnormal behaviours. The behavioral changes showed by fishes as a result of exposure to Copper oxychloride included 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 (Table 1).

Table 1. Behavioural changes in different fish groups exposed to pesticides

Behaviour	24 Hours	48 Hours	72 Hours	96 Hours
Irregular Swimming	✓	✓	✓	✓
Loss of body equilibrium	✗	✗	✓	✓
Jerky Movements	✓	✓	✗	✗
Altered feeding behaviours	✗	✓	✓	✓
Altered social behaviours	✗	✓	✓	✓
Hypersecretion of mucus	✗	✗	✓	✓
Increased opercular movements	✓	✓	✓	✓
Opening of the mouth before death	✓	✓	✓	✓
Surface or bottom dwellers	✗	✓	✓	✓
Loss of body scales	✗	✗	✓	✓
Changes in body colouration	✗	✗	✗	✓
Bending of fins	✗	✗	✗	✓

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 75 mg/l, 60 mg/l, 20 mg/l and 12 mg/l. The LC50 (sub-lethal concentration) for 24, 48, 72 and 96 hours were 125 mg/l, 95 mg/l, 60 mg/l and 50 mg/l. The LC100 (Lethal concentration) values for 24, 48, 72 and 96 hours were 190 mg/l, 125 mg/l, 95 mg/l and 60 mg/l (Table 2).

Table 2. LC values of copper oxychloride to *Carassius auratus* for different periods

S. No	Exposure time	LC0 Value (mg/L)	LC50 Value (mg/L)	LC100 Value (mg/L)	P value
1	24 hours	75	125	190	0.001
2	48 hours	60	95	125	0.001
3	72 hours	20	60	95	0.001
4	96 hours	12	50		60

Discussion

In the current study, the *Carassius auratus* (Goldfish) were exposed to different concentrations of copper oxychloride (100%) to find out LC0, LC50 & LC100 for 24, 48, 72 & 96 hours. The LC0 for 24, 48, 72 and 96 hours were 75 mg/l, 60 mg/l, 20 mg/l and 12 mg/l. The LC50 for 24, 48, 72 and 96 hours were 125 mg/l, 95 mg/l, 60 mg/l and 50 mg/l. The LC100 for 24, 48, 72 and 96 hours were 190 mg/l, 125 mg/l, 95 mg/l and 60 mg/l. The abnormal behaviours in exposed fish to pesticides 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 the water surface or becoming bottom dwellers. As the concentrations of pesticide toxicants increased, the time of exposure decreased, and the rate of mortality increased.

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 current study. The current study reported the safe, sub and lethal concentrations of Cobox (Copper oxychloride 100%) to wild *Carasius auratus*; the LC0 for 24, 48, 72 and 96 hours were 75, 60, 20, and 12 mg/l. The LC50s were 125, 95, 60, and 50 mg/l. The LC100 values were 190, 125, 95, and 60 mg/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 observed in the current study.

A study was done to determine the LC50 value of the insecticide Chlorpyrifos on the fingerlings of Common carp (*Cyprinus carpio*) for 96 hours, which was 0.160 mg/L. In our present study, we have taken different fish *Carassius auratus* commonly known as goldfish, which were exposed to different pesticides, Copper oxychloride, for which the LC50 for 24, 48, 72 and 96 hours were 125 mg/l, 95 mg/l, 60 mg/l and 50 mg/l. The behavioural changes induced by copper oxychloride on *Carassius auratus* in our experiment were similar to the previous study, which also identified some of the morphological changes, such as caudal bending and reported that these morphological and behavioural changes may be due to inhibition of the enzyme Acetylcholinesterase. However, the LC50 values differed between the two studies. So, it is concluded that Chlorpyrifos is more toxic than Copper oxychloride, and the *Carassius auratus* fish is more resistant than the *Cyprinus carpio*.

A study was also conducted by Singh, Pandey et al. (2009), in which they exposed fingerlings of common carp to the organophosphate pesticide dimethoate to determine the LC50 values for 24, 48, 72, and 96 hours and the behavioural abnormalities caused by the pesticide. The LC50 values 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 pesticide exposure 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 125 mg/l, 95 mg/l, 60 mg/l and 50 mg/l. The behavioural changes induced by the pesticides in both studies are 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 similar to the present study. While they reported that decreased opercular movement rate was not observed in the current work but instead the opercular movements were increased in the current study.

Research was done by (Ezeonyejiaku, Obiakor et al. 2011), in which they determined the acute toxicity LC50 of Copper sulphate on two fish species, Tilapia and Catfish, exposed for 96 hours. The LC50 values of copper sulphates for Tilapia and Catfish for 96 hours were quite higher (58.837 and 70.135 mg/l, respectively) than the LC50 values for 96 hours obtained in the current experiment. In the current study, the LC50 for 24, 48, 72 and 96 hours were 125 mg/l, 95 mg/l, 60 mg/l and 50 mg/l. The behavioural changes induced by copper oxychloride in

our study and Copper sulphate in the previous study are similar in both studies. It is concluded that excessive Copper in water bodies has harmful effects on the behaviours and mortality of fish.

A work was also done to determine the LC50 of pesticide Copper sulphate on the fingerlings of Nile tilapia fish for 96 hours of exposure. The LC50 value determined in their research study was 31.2 mg/l. In the present study, the LC50 value of copper oxychloride for 96 hours on *Carassius auratus* was 50 mg/l. Their study supports the current work as the behavioural changes caused by copper sulphate in the previous study and Copper oxychloride in our 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 like the present study.

Conclusion

From the present study, it is concluded that;

1. Cobox (Copper oxychloride) fungicide is toxic to *Carasius auratus,* and high concentrations caused mortality in them.
2. It also adversely affected their behaviours.

Changes in behaviour included irregular swimming, jerky movements, altered feeding and social behaviours, loss of equilibrium and loss of body scales.

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Author's contributions

AA, FAK and MAK collected samples and did experimental work; NU and SI formulated the research and wrote the manuscript; AR, HUR & MY formulated the results.

Conflict of interest

The authors have no conflict of interest. All authors agree to publish the paper.

Recommendations

The use of safer pest control strategies should be fostered, while chemical broad-spectrum pesticides should be avoided.

Innovation/Cutting-Edge Research

In the current research work, the lethal concentrations of Copper oxychloride pesticide are determined for *Salmo trutta fario* that can cause mortalities in trout. Alongside the behavioural responses was also measured as response to the Copper oxychloride pesticide. According to our best knowledge, no such work was done in District Swat before.

Research Location

The research was carried out in the Centre for Animal Sciences & Fisheries, University of Swat, Pakistan.

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