Toxicity Assessment of Hexaconazole to a Fish (Channa punctatus, Bloch) using certain Hepatic Biomarker Enzymes

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KEYWORDS

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Abstract

Hexaconazole (5% EC) is a broad-spectrum fungicide with long-term ecotoxicological effects, posing a risk to the environment and human health. It treats many fungal infections in crop fields. The study assesses the acute toxicity and biochemical changes in Channa punctatus exposed to the fungicide hexaconazole. Within the acute toxicity test, 96hr-LC50 values of hexaconazole of 80.0 mg/l. Hepatic biomarker enzymes such as alkaline phosphatase and alanine transaminase levels were dose and duration-dependent increases/decreases. The changes within the hepatic biomarker enzymes in Channa punctatus are warning indications that their existence in their natural environment seems jeopardized. Hence, the current study reveals that the employment of hexaconazole was classified among substances harmful to fish. The build-up of the fungicide within the muscle tissue in fishes might be hazardous to living organisms.

INTRODUCTION

Fungicides prevent and treat fungal diseases in crops, plants, and other agricultural products. Their use is critical for guaranteeing food security, preserving crop productivity, and minimizing economic losses caused by fungal diseases. Fungicides are used in agriculture, horticulture, and forestry through spraying, seed treatment, or soil incorporation.

However, the widespread use of fungicides has prompted serious environmental issues, particularly the impact on aquatic habitats. Following application, fungicides may infiltrate surrounding bodies of water by surface runoff, leaching, spray drift, or incorrect disposal. Once in marine settings, these compounds may persist and accumulate, providing dangers for a wide range of underwater creatures, including algae, invertebrates, fish, and amphibians.

Many fungicides are harmful to non-target species and can disrupt ecological processes. For example, some fungicides disrupt aquatic organisms' reproduction, growth, and behavior, whilst others may destroy biodiversity by preferentially targeting sensitive species. Fungicides can also change microbial communities and nutrient cycling, potentially disrupting the balance of aquatic ecosystems.

Fish are among the most sensitive aquatic creatures to the effects of fungicides in water bodies. These pollutants can infiltrate freshwater and marine environments via agricultural runoff, wastewater discharge, and unintentional spills, causing acute and chronic harmful effects on fish populations.

Fish exposed to fungicides exhibit behavioral changes such as reduced eating, irregular swimming, and impaired predator avoidance. These modifications may reduce survival rates and upset the natural equilibrium of aquatic food webs.

Fungicides can disrupt physiological processes in fish. Fungicide exposure can decrease liver enzyme activity and cause histological abnormalities in the liver and kidney tissues. Some fungicides mimic or inhibit hormone activity, influencing reproduction and growth. This can lead to lower fertility, changed sex ratios, and

developmental defects in kids. Prolonged exposure to fungicides may decrease the fish's immunological system, making them more vulnerable to infections and other environmental stressors.
Certain fungicides bioaccumulate in fish tissues, endangering not only the fish but also predators such as birds, animals, and humans consume them. It can have long-term ecological and health repercussions at multiple trophic levels.

Many fungicides, notably synthetic ones like propiconazole, tebuconazole, hexaconazole, and mancozeb, are known to be hazardous to fish even at low levels. Acute exposure can cause respiratory distress, loss of equilibrium, aberrant swimming behavior, and possibly death. The level of toxicity varies according to the fish's species, age, and developmental stage.

Many researchers investigated the impact of pesticides on fish and discovered distinct changes in liver enzymes. Rao[(1)] reported an increase in acyl carrier protein and alkaline phosphatase activities in plasma, kidney, and gills, as well as a significant decrease in both activities in liver tissues, as a result of monocrotophos exposure in Oreochromis mossambicus. Koul et al. [(2)] and Mastan and Ramayya [(3)] recorded biochemical alterations in Channa gachua after exposure to dichlorvos and noted an increase in alkaline phosphatase in plasma, Serum glutamate pyruvate transaminase (alanine transaminase), and serum glutamate oxaloacetate transaminase (aspartate aminotransaminase) in both acute and chronic studies. Karami-Mohajeri and Abdollahi [(4)] discovered liver damage in Clarias batrachus exposed to phorate and carbaryl, which cause the production of lipolytic mitochondrial enzymes that dissolve lysosomal membranes, cell membranes, and hepatocellular organelles, releasing free liver enzymes into the bloodstream. Bhavika [(5)] discovered a significant increase in alkaline phosphatase levels in the liver, kidney, gills, and muscle of Oreochromis mossambicus and Labeo rohita following imidacloprid and curzate administration. Increased alkaline phosphatase levels may indicate hepatic and kidney impairment [(6, 7)]. Rahman et al. [(8)] found that profenofos treatment significantly increased blood levels of alanine transaminase, aspartate aminotransaminase, and alkaline phosphatase in Cyprinus carpio.

Excessive usage of these compounds hurts the environment, damaging terrestrial and aquatic species and disturbing the ecological balance, making them a possible hazard to the ecosystem. So, we will investigate hexaconazole toxicity in Channa punctatus because it is widely disseminated and consumed in South Asia. It is a bottom feeder, active all year, and is sensitive to environmental changes [(9).] The Indian government also promotes the commercial production of murrel fish. We investigated the effects of sublethal hexaconazole concentrations on the liver of Channa punctatus by assessing biomarkers of liver injury such as alkaline phosphatase and aspartate transaminase.

2. Materials and Methods

2.1. Experimental Layout

Channa punctatus (body weight: 25-30 gm total length: 12-14 cm) were collected from local fishermen of Arrah during the season of fish during 2023. The fishes were disinfected with dilute $KMnO_4$ and then transferred to large aquaria. The fishes were fed with pieces of goat liver and fish food available in the local market.

2.2. Density of fish

The investigation was carried out in a controlled laboratory setting using a static renewable method by the ethics of the Department and VKS University, Arrah. Temperature (26.0 ± 2.0^0C), pH (7.14 ± 0.08), dissolved oxygen (6.4 ± 0.64mg/L), total alkalinity (54.00 ± 8.42mg/L) and total hardness (150. 6 ± 10.66mg/L) were measured daily at exposure times of 24, 48, 72 and 96 hours [(10)]. According to Muirhed Thomson [(11)] and Holden [(12)], LC50 maintained a constant ratio of fish biomass to water volume by using 1 g weight/1 litre of water to minimize differences in toxicity owing to density and oxygen depletion.

2.3. Test Chemical

Hexaconazole (5% EC; Molecular formula: $C_{14}H_{17}Cl_{12}N_3O$; Molecular mass: 314.21g/mol), a technical grade broad spectrum insecticide manufactured by KBA, Surat, Gujarat, India, was taken for evaluation of its toxicity.

2.4. Acute Study

The fatality rate was monitored and reported at 24-hour, 48-hour, 72-hour, and 96-hour intervals. Finney's [13] approach was used to calculate the LC50, in which the probit mortality was plotted against the log of fungicides.From this value, 1% and 5% of 96h-LC50 dose was used for serum biochemical studies.

OBSERVATIONS AND RESULTS

(A) Finney's [13] probit analysis method: It is a parametric maximum likelihood method that calculated net/corrected percent mortality from 10% to 100% after calculating percent mortality. Then, from the Fischer and Yates table, values of empirical probit ranging from 3.72 to 8.72 (Table 2) were noted based on the straight line obtained in the graph. Expected/provisional probit was calculated using empirical probit values ranging from 4.40 to 6.80. To determine the mean and deviation of the hexaconazole dose and mortality, the values of the working probit (from 3.81 to 7.45) and weighing coefficient (from 0.248 to 0.547) were calculated. Finally, the median lethal concentration of hexaconazole was calculated to be 96hr-LC_{50} = Antilog 1.903 = 80.0 mg/L. Because the calculated value of χ^2 is less than the tabulated value, the data is homogeneous, indicating that the 'eye fit' line is in good agreement. After determining the value of 96hr-LC50 of hexaconazole to be 80.0 mg/l, a concentration of 0.80 mg/l (1% of 96hr-LC50) and 4.00mg/l (5% of 96hr-LC50) of hexaconazole was selected for this study. In other words, in this experiment, 0.80mg and 4.00mg of hexaconazole were applied for the intoxication of fish.

Table 1: Toxicity classification of different fungicides.

Toxicity classification	**LC50 (mg/l*)**	**Toxicity classification**	**LC50 (mg/l*)**
Super	<0.01	Moderate	1.1 to 10
Extreme	0.01 to 0.10	Slight	11-100
High	0.11 to 1.0	Minimal or Non-toxic	>100

Table 2: Probit analysis for toxicity of Hexaconazole (5% EC) in Channa punctatus.

Dose of Hexaconazole (5% EC)	Log dose of Hexaconazole	Number of fish exposed	Mortality of fish	% of mortality of fish	Net/corrected mortality of fish	Empirical probit	Expected /Provisional probit	Working probit	Weighing Coefficient	w	wx	nwy	nwx^2	nwy^2	nwxy
	x	n		p			Y	y	w						
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	1.477	20	2	10	10	3.72	4.40	3.81	0.246	4.92	7.27	18.75	10.73	71.44	27.70
60	1.778	20	7	35	35	4.51	5.60	4.42	0.505	10.10	17.96	44.64	31.93	197.31	79.38
90	1.954	20	12	60	60	5.25	6.00	5.26	0.548	10.96	21.44	57.65	41.85	303.24	112.77
120	2.079	20	18	90	90	6.28	6.40	6.12	0.514	10.68	22.58	66.46	46.94	406.74	138.19
150	2.176	20	20	100	100	8.72	6.80	7.45	0.587	11.74	25.55	75.72	55.59	488.39	162.86
	-	-	-	-	-	-	-	-	-	48.58	94.8	263.22	187.04	1467.12	520.90

Following calculations made from the table:
(a) $\bar{x}$ = 1.95, (b) $\bar{y}$ = 5.42, (c) S_{xx}= 2.05, (d) S_{yy}= 40.92, (e) S_{xy}= 7.25, (f) χ^2= 15.28 (tabulated value 16.266 at p=0.001 and df = 4), (g) b = 19.96 and (h) S = 1.105.

96hr-LC_{50} = Antilog 1.903 = 80.00mg/l (0.8000ppm) of Hexaconazole (5% EC).

(B) In a study examining the effects of hexaconazole on the serum alkaline phosphatase levels in Channa punctatus, fish were exposed to a sub-lethal concentration of 0.80 mg/l for varying durations. Short-term exposure (24 to 96 hours) resulted in slight increases in enzyme levels compared to the control, though these changes were statistically insignificant. Conversely, after chronic exposure periods (15 to 60 days), a significant decrease in alkaline phosphatase levels was observed, these reductions were statistically significant. Analyses indicated that variations in hexaconazole dosage and exposure duration did not significantly influence serum alkaline phosphatase levels (Table 3).

Exposure of fish to 4.0 mg/L hexaconazole resulted in variable effects on serum alkaline phosphatase levels over different timeframes. At 24, 48, and 96 hours, values showed a slight increase compared to control, but these changes were statistically insignificant. Conversely prolonged exposure for 15, 30, and 60 days; led to notable decreases in alkaline phosphatase levels, indicating a moderately significant decline over time. Statistical analysis revealed that the dosage and duration of exposure had no significant influence on alkaline phosphatase levels in Channa punctatus (Table 3).

Exposure to hexaconazole significantly reduced the serum alkaline phosphatase levels in Channa punctatus, with average decreases to 54.98, 55.36, and 55.22 iu/l, representing a decline of around 1.27% to 1.96% from a control level of 56.08 iu/l. Statistical analysis confirmed a significant effect of hexaconazole dosage (F=4.79, p<0.05) on serum alkaline phosphatase levels of differing exposure durations did not have a statistically significant impact (F=0.061, p>0.05).

Table – 3. Variations in serum alkaline phosphatases (iu/l) of Channa punctatus after acute and chronic exposure of two sub-lethal doses of Hexaconazole (5% EC)

Dose of Hexaconazole (5% EC) (mg/l)	Controlled value and Range	Duration of exposure			t-test	F value
		a=24 hours b=15 days	a=48 hours b=30 days	a=96 hours b=60 days		
0.80	56.08±6.63 (20.0 – 200.0)	56.87±5.27 a (+1.41%) NS	58.15±3.52 a (+3.69%) NS	59.74±5.16 a (+6.53%) *	2.62^{NS}	$F_{1,3} = 4.38^{NS}$ $F_{3.3} = 0.9^{NS}$
		53.60±5.48 b (-4.42%) NS	46.60±3.54 b (-16.90%) ***	50.87±3.61 b (-9.29%) *	2.81*	
4.00		57.47±3.88 a (+2.48%) NS	61.84±3.50 a (+10.27%) **	65.23±3.59 a (+16.32%) ***	2.41^{NS}	$F_{1,3} = 5.76^{NS}$ $F_{3.3} = 0.12^{NS}$
		51.98±3.54 b (-7.31%) *	49.88±2.49 b (-11.06%) **	50.01±2.62 b (-10.82%) **	8.03*	
Average		**54.98±2.63 (-1.96%) NS**	**55.36±8.63 (-1.28%) NS**	**55.22±5.73 (-1.77%) NS**	**8.05***	$F_{3,9} = 4.79^*$ $F_{3,9} = 0.061^{NS}$

(NS= Not Significant, *= Significant, **= Moderately Significant, ***= Highly Significant).

(C) Serum alanine transaminase plays a crucial role in amino acid metabolism, facilitating the conversion between alanine and α-ketoglutarate, resulting in pyruvate and glutamate formation. Its activity is sourced from

the liver as a vital biochemical marker for evaluating liver integrity and function in fish, particularly in aquatic toxicology and fish health monitoring.

Exposure to 0.80 mg/l of hexaconazole in fish (Channa punctatus) significantly increased serum alanine transaminase (ALT) levels in the short term, with values rising by approximately 11.81% to 20.69% over 24 to 96 hours compared to controls. However, after prolonged exposure (15, 30, and 60 days), ALT levels decreased, showing depletions ranging from 5.70% to 38.66%. Statistical analysis revealed that the variation in hexaconazole dosage and the duration of exposure had no significant effects on ALT levels, indicating that while short-term exposure affected enzyme activity, long-term consequences were minimal (Table 4).

Fish exposed to 4.0 mg/L hexaconazole showed an increase in serum alanine transaminase (ALT) values over short durations (24 to 96 hours), with changes of +15.31% to +18.70% compared to controls, indicating highly significant short-term effects. However, after chronic exposure (15 to 60 days), ALT levels decreased significantly, declining by 20.64% to 29.94%. Despite these alterations, neither hexaconazole dose variation nor exposure duration significantly affected serum alkaline transaminase levels in Channa punctatus (Table 4).

Exposure to hexaconazole resulted in varying serum alanine transaminase levels in Channa punctatus, with changes recorded as -3.50%, -9.56%, and +2.82% compared to the control. While the overall effect of hexaconazole on alanine transaminase was found to be insignificant ($t = -0.95$, $p > 0.05$), a significant influence of hexaconazole dose variation ($F=6.89$, $p<0.05$), suggesting that the dosage plays a critical role in affecting enzyme levels. Conversely, the duration of exposure showed minimal influence on serum alanine transaminase levels ($F=0.71$, $p>0.05$).

Table – 4. Variations in serum alanine transaminase (iu/l) of Channa punctatus after acute and chronic exposure of two sub-lethal doses of Hexaconazole (5% EC)

Dose of Hexaconazole (5% EC) (mg/l)	Controlled value and Range	Duration of exposure			t-test	F value
		a=24 hours b=15 days	a=48 hours b=30 days	a=96 hours b=60 days		
0.80	19.14±3.5 (5.0 – 90.0)	21.00±1.74 (+9.71%) *	21.60±1.80 (+12.85%) **	23.10±1.78 [a] (+20.69%) ***	5.39*	$F_{1,3} = 6.41^{NS}$ $F_{3.3} = 0.64^{NS}$
		15.65±1.76 (-18.23%) ***	11.90±1.96 [b] (-38.66%) ***	18.05±1.82 [b] (-5.70%) *	2.19^{NS}	
4.00		22.07±1.74[a] (+15.31%) ***	22.34±1.88 [a] (+16.76%) ***	22.72±1.70 [a] (+18.70%) ***	17.17**	$F_{1,3} = 8.61^{NS}$ $F_{3.3} = 0.07^{NS}$
		15.19±1.86 [b] (-20.64%) ***	13.41±1.94 [b] (-29.94%) ***	14.86±1.78[b] (-22.36%) ***	8.52*	
Average		**18.47±3.56 (-3.50%) NS**	**17.31±5.42 (-9.56%) ***	**19.68±3.95 (+2.82%) NS**	**0.95^{NS}**	$F_{3,9} = 6.89^{*}$ $F_{3,9} = 0.71^{NS}$

(NS= Not Significant, *= Significant, **= Moderately Significant, ***= Highly Significant).

DISCUSSION

A fungicide's capacity to harm fish and aquatic animals follows its toxicity, exposure time, dose rate, and persistence in the environment. Table 1 presents the toxicity classification of fungicides based on LC50 values.

Hexaconazole treats numerous infections brought about by ascomycetes, basidiomycetes, and blemished growths. It is especially suitable to treat growth illnesses like buildup, rust, scab, earthy-colored smudge, and anthracnose brought about by the ascomycetes and basidiomycetes [(14)].

Wheeler [15] developed a formula for calculating the percent disease index (PDI) and dose of the most commonly used fungicides. Hexaconazole (2.00-2.50 ml/liter) is roughly three times more potent than Tricyclazole (0.60-0.75 ml/liter) in PDI. Pandit and Rani [16] determined the average 96hr-LC50 tricyclazole dose for Channa punctatus to be 25.0 mg/L. Based on the toxicity table, hexaconazole seems to be a slightly toxic fungicide for fish.

Choudhury et al. [17] determined 96hr-LC50 of 25.003mg/l of Contaf (hexaconazole). The current work OF hexaconazole for 96hr-LC50 = 80.0 mg/L is consistent with the observations of Wheeler [15] and Pandit and Rani [16]. Furthermore, a safe level of hexaconazole is 0.080 to 0.80 mg/L for rats and 0.0080 to 0.080 mg/L for humans (https://en.wikipedia.org/wiki/Toxicity). So far, India has accepted the shipment of paddy with a Trizole maximum residue limit (MRL) of 0.03 mg/kg. At this dose, the rat and human-safe dose of hexaconazole exceeded.

Alkaline phosphatase hydrolyzes phosphate esters and is essential for metabolic processes such as bone mineralization and liver function. Its activity can be altered by pesticide exposure, with increases indicating liver damage or stress from fungicide toxicity, which may signal tissue repair. However, prolonged exposure may decrease alkaline phosphatase activity due to enzyme inhibition and liver damage. In aquaculture, serum alkaline phosphatase is used as a biochemical marker to evaluate organ function and fish health, as its levels reflect physiological stress, environmental pollutants, nutritional problems, or diseases.

Increased serum alanine transaminase levels are a crucial non-lethal biomarker for assessing liver stress and health in fish, particularly in aquaculture and ecotoxicological studies. These levels can rise significantly following acute exposure to toxic factors, such as hexaconazole, indicating hepatocyte damage and subsequent enzyme leakage into the bloodstream. However, variations in alanine transaminase activity arise from species-specific responses and environmental influences, necessitating careful interpretation. Over time, elevated alanine transaminase levels may decrease as liver function deteriorates, reflecting the compromised ability of hepatocytes to synthesize and release the enzyme.

Our study is consistent with the findings of other studies in fish. Ayanda et al. [18] similarly showed that alanine transaminase and alkaline phosphatase significantly elevated in Channa gariepinus. Khan et al. [19] observed significant ($p < 0.05$) increases in liver biomarkers (alanine transaminase, alkaline phosphatase) after 20, 40, and 60 days of mancozeb exposure in Channa punctatus. Alkaline phosphatase and alanine transaminase are liver injury biomarkers enzymes [20]. Liver biomarker enzymes like alkaline phosphatase and alanine transaminase catalyze transamination reactions and, in the detection and differential etiologic diagnosis of hepatic disease. Fluctuations in their concentrations can be an index of liver injury and tissue health.

The activities of alkaline phosphatase and alanine transaminase significantly elevated after exposure to chlorfenapyr, dimethoate, and acetamiprid in the investigation of Ghayyur et al. [21]. In the study of Esenowo et al. [22], there was a significant increase in the alanine transaminase and alkaline phosphatase levels of African catfish, Clarias gariepinus, exposed to chlorfenapyr in comparison with the control, and acute exposure to chlorfenapyr can change liver enzyme activities and serum lipid profile, initiating a jump in the energy requirement of the exposed organism.

CONCLUSION

The current work shows that exposure to sub-lethal dose of hexaconazole, a triazole fungicide, drastically changes the alkaline phosphatase and alanine transaminase in Channa punctatus. With increasing doses and durations of hexaconazole exposure, alkaline phosphatase and alanine transaminase activity in critical tissues such as the liver, kidney, and gills increased, and then decreased. The increase, followed by a decrease in alkaline phosphatase activity, reflects hexaconazole's inhibitory effects on protein synthesis and phosphate metabolism, which are necessary for normal physiological activities. As a result, alkaline phosphatase and alanine transaminase are accurate biochemical biomarkers for determining the sublethal toxic effects of hexaconazole in aquatic creatures. These findings illustrate the ecological dangers associated with indiscriminate pesticide use, emphasizing the importance of regulated application to safeguard aquatic/marine life.

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