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Acute Toxicity of Hexaconazole on Freshwater Fish *Channa punctatus* (Bloch)

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Research Article

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ABSTRACT

The present study was carried out to investigate the lethal toxicity and stress of commercial formulations of hexaconazole, ((RS)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl) hexan-2-ol) fungicide, toward freshwater fish *Channa punctatus* (Bloch). Estimated 96-hour LC_{50} of hexaconazole was found to be 103.27 µl/L. On the basis of the obtained LC_{50} values for 96-hour exposure intervals, hexaconazole can be rated as highly toxic to *C. punctatus*, with significant changes observed behaviour of fishes. The estimated safe levels showed large variations for the test pesticide.

Keywords: Lethal toxicity; Hexaconazole; *C. Punctatus*; LC_{50}; Safe levels

INTRODUCTION

Pesticides are unique, intrinsically hazardous chemicals designed to be deliberately spread into the environment to kill off pests and pose particular problems for human health and the environment [1]. Approximately 5.2 billion pounds were used worldwide in 2006 and a similar amount in 2007 but only 1% of the volume actually reaches the desired targeted pests at lethal doses. In India, as much as 70% of chemical formulations, employed in agricultural practices, are believed to affect nontarget organisms and find their way to freshwater bodies [2].

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Hexaconazole, [(RS)-2-(2, 4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2-ol], is a widely used triazole fungicide used in crop protection *viz* cereals, vegetables, field crops, and fruit (primarily orchards) [3]. Hexaconazole is effective against many fungi (particularly basidiomycetes and ascomycetes fungus). The mechanism is inhibiting by the biosynthesis of ergosterol to prevent fungal mycelium development [4]. In addition, the enantioselective degradation of hexaconazole in cucumber, tomato, soil, rabbit and rat hepatic microsomes was researched, and the (+) -hexaconazole was preferentially dissipated. Adverse effects of hexaconazole on pregnancy and its outcome and circulating levels of estradiol and progesterone were reported by Kumar et al [5].

Fishes are being used as useful models for the evaluation of ecotoxicity in aquatic ecosystems. Ecotoxicological characteristics fish *Channa punctatus* such as its wide distribution and availability throughout the year, easy maintenance in the aquaria/wet lab, and commercial importance make this species an excellent model for toxicity studies [6]. The effects of toxicity are reported to be several-folds on fitness traits and subsequent population dynamics in fish have been highlighted during toxicity assessment experiments [7].

Since there is growing concern over the presence of toxicity in the aquatic environment, it is important to develop methods for detection of toxic effects in aquatic organisms [8]. Freshwater fish *Channa punctatus*, species is commonly found in India, Pakistan, Bangladesh, and other countries [9]. It fulfils most of the requirements of a model species, including availability, throughout the year [10].

Under laboratory conditions, toxicity testing procedures (mortality studies LC_{50} estimates) provide information regarding the harmfulness of industrial stress for aquatic animals, including fishes [11]. In the acute toxicity of contaminants in static bioassays, the use of 96 h, LC_{50} has been widely recommended as a preliminary step in toxicological studies on fishes [12]. Therefore, in view of the ecological impact of this fungicide, the present study aimed at a determination of 96-h LC_{50} of hexaconazole, and their behavioral responses during exposureto *C. punctatus* [13].

MATERIALS AND METHODS

Experimental fish specimens

The freshwater fish *C. punctatus* (Bloch) belongs to family: Channidae and order: Perciformes, was obtained from the local sources with an average body size of 12.0 ± 4.0 cm and body weight of 20 ±2.5 g at the juvenile life stage and acclimated in laboratory conditions for 10 days before study [14]. They were acclimated in the laboratory condition for 10 days before experimentation. They were kept in glass aquaria during the acclimatization. Fish were fed on fish food at the rate of approximately 4% of fish body weight [15]. The photoperiod was maintained as per normal day and night approximately 12 hours, and every effort was made to provide optimal condition for fish; no mortality occurred during this period [16].

Test chemical

Before exposure, quality of water was tested according to the APHA, AWWA, WEF. The fungicide for the study, hexaconazole (5% SC), with the product name of Comfort plus (Anu Products Ltd., India), was purchased from a local market. The test chemical structure and specifications are summarized in Figure 1 and Table 1 [17].

Figure 1. Structure of Hexaconazole.

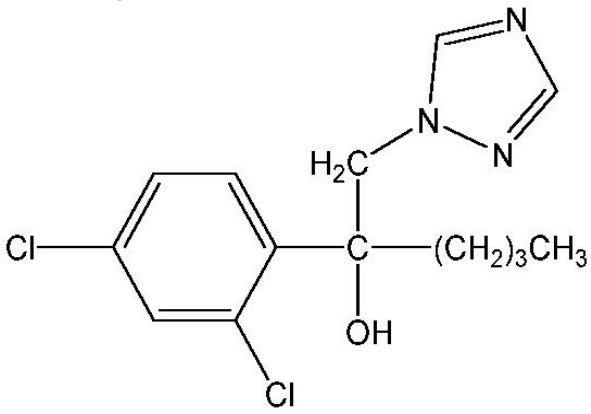

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Table 1. Characteristics/properties/specification of the test chemical.

Product name/Common name	Hexaconazole
Classification	Fungicide
Chemical/product name	Comfort plus
Chemical name	(RS)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2-ol
Class	Triazole fungicide
CAS number	79983-71-4
Supplier	Anu products LTD., India
Toxicity class	WHO (a.i.) U; EPA (formulation) IV

Acute toxicity bioassay

The acute toxicity bioassay was conducted in a static system to determine the 96-h LC_{50} value of hexaconazole. Test concentrations were determined on the basis of the range-finding assay, where the lowest value was selected as the highest concentration at which 0% mortality occurred and the highest value as lowest concentration at which 100% mortality occurred [18]. The test specimens were exposed to six different nominal concentrations (60, 80, 100, 120, 140, and 160, µg/L), along with one control (without test chemical), keeping 10 fish in each test concentration in 20 L of water in glass aquaria without change of water [19]. No crowding stress was observed during experimentation. Mortality of fish from hexaconazole exposure was recorded up to 96 hours at every 24-hour interval. Feeding was stopped 24 hours before exposure, and fish were not fed during the experimentation period, as recommended by Pandey et al., No mortality was observed in the control group of fish [20]. Fish behavior was observed during the exposure. For the determination of LC_{50} value and 95% confidence limits of the LC_{50} values of hexaconazole, was calculated from the data obtained in acute toxicity bioassays SPSS (version 16) software. The safe level estimation after 96 h exposure for fungicide was based on Sprague, CWQC, NAS/NAE, and IJC.

RESULTS

Physicochemical parameters of the test water

Physicochemical parameters were measured before fish loading and during experimentation. The temperature of the test water varied from 18.3°C to 21.6°C and pH value ranged from 7.1 to 7.8. The Dissolved Oxygen (DO) ranged from 6.9 to7.8 mgL^{-1}. The conductivity of the water ranged from 248 to 305 µM cm^{-1}, whereas total hardness ranged from 184 to 240 mgL^{-1} during the experiment. The physicochemical parameters of the test water are presented in Table 2.

Table 2. Physicochemical parameters of the test water.

Sr. No.	Parameter	Unit	Mean	Range
1	Air temperature	°C	24.8	23-26
2	Water temperature	°C	19.6	18.3-21.6
3	pH		7.5	7.1-7.8
4	Dissolved oxygen	mgL^{-1}	7.1	6.9-7.8
5	Conductivity	μMcm^{-1},	279	248-305
6	Total hardness	mgL^{-1}	190	184- 240

Behavioral response and poisoning symptoms in fish to test concentration

The fish, *C. punctatus* exposed to hexaconazole expressed behavioural abnormalities, the elucidated 96 h LC_{50} value indicated high toxicity of the test chemical, which induced abnormal such as erratic swimming, loss of equilibrium, increased surface activity, enhanced surfacing, rapid opercular movement, and secretion of mucous from the whole body of the test species. At the beginning of exposure, fish were found to be healthy and very active. During the experiment, they tried to avoid the test water for sometime by swimming fast, jumping, and other random movements in treated groups. At higher test concentrations, fish expressed erratic swimming with jerky movements, along with hyper excitation. With the continuation of the exposure, the fishes progressively became sluggish and lethargic. Under such conditions, efficiency of oxygen uptake decreased considerably, which was manifested as enhanced breathing rate along with more frequent visits to

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surface water for gulping fresh air. Eventually, there was loss of balance, exhaustion, and lethargy owing to respiratory incumbency. At last, the fish struggled hard for breathing some time engulfing atmospheric air and avoided to toxic medium. Soon, they settled at the bottom of the tank; and after bellies turned upward and the fish died.

Median lethal concentration and application factor

The acute toxicity bioassays to determine the LC_{50} 96 h value of hexaconazole were conducted in the semi-static system. The LC_{50} 96 h value of hexaconazole was determined as 103.27µl/L for *C. punctatus* using SPSS software (version 16). The LC_{50} values (with 95% confidence limits) of different concentrations of hexaconazole were 192.42 (157.26-879.02), 148.88 (133.15-184.73), 130.15 (113.54-156.72) and 103.27 (87.81-117.72) µl L^{-1} for 24, 48, 72 and 96 h, respectively. Acute toxicity bioassays, of different lethal concentrations (with 95% confidence limits) at different exposures are listed in Table 3. The safe levels of hexaconazole at 96 h LC_{50} value based and using with different "Application Factors (AF)" are listed in Table 4. There were variations in safe levels estimated by different methods at 96 h of fish. The safe level values of hexaconazole in *C. punctatus* under estimation varied from 10.327 to 1.0327 x 10^{-3} µl L^{-1}.

Table 3. Lethal concentrations of hexaconazole (with 95% confidence intervals) depending on exposure time for *C. punctatus*.

Lethal concentration	Exposure times (h)			
	24	48	72	96
LC_{10}	123.11 (2.03-160.09)	99.67 (55.47-116.48)	70.26 (18.24-91.01)	56.18 (13.93-75.06)
LC_{20}	146.9 (118.99-343.22)	116.56 (89.84-132.20)	90.82 (56.66-107.86)	72.35 (41.19-87.81)
LC_{30}	164.06 (137.71-540.88)	128.74 (110.25-147.92)	105.65 (81.79-122.58)	84 (60.03-97.81)
LC_{40}	178.72 (148.49-714.98)	139.15 (123.48-165.56)	118.31 (99.92-138.51)	93.96 (75.09-107.39)
LC_{50}	192.42 (157.26-879.02)	148.88 (133.15-184.73)	130.15 (113.54-156.72)	103.27 (87.81-117.72)
LC_{60}	206.12 (165.45-1043.64)	158.61 (141.38-205.35)	141.99 (124.79-177.30)	112.58 (98.88-129.69)
LC_{70}	220.78 (173.87-1220.11)	169.02 (149.35-228.25)	154.66 (135.33-200.81)	122.54 (109.04-144.17)
LC_{80}	237.94 (183.45-1426.91)	181.2 (158.11-255.61)	169.48 (146.67-229.33)	134.19 (119.45-162.61)
LC_{90}	261.73 (196.46-1713.98)	198.1 (169.73-294.09)	190.04 (161.53-269.74)	150.36 (132.47-189.59)

Table 4. Estimation of safe levels of hexaconazole at 96 h exposure duration in *C. punctatus*.

Chemical	96 h LC_{50} (µl L^{-1})	Method	AF	Safe level
Hexaconazole	103.27	Sprague (1971)	0.1	10.327
		CWQC (1972)	0.01	1.0327
		NAS/NAE (1973)	0.1–0.00001	10.327-1.0327 x 10^{-3}
		IJC (1977)	5% of 96 h LC_{50}	5.1635

DISCUSSION

Occurrence of pesticides and fungicides in high concentrations in agricultural wastewaters and their toxicity to aquatic fauna especially fish species have been reported by many researchers. Fish are often used as sentinel organisms for ecotoxicological studies because they play number of roles in the trophic web, accumulate toxic substances and respond to low concentration of toxicity. Serious concerns remains due to their potential to cause adverse effects on human and wildlife populations, and environments. Therefore, the importance of uses of fish as bio-indicators of the effects of pollution is increasing and can permit early detection of aquatic environmental problems.

The purpose of this study was to investigate the acute toxicity of hexaconazole on the Indian freshwater fish, *C. punctatus*. The test result of the 96-hour LC_{50} value of hexaconazole was determined to be 103.27 µl/L, which indicated hexaconazole to be very toxic. To the best of our knowledge, no literature is available on the acute toxicity of hexaconazole to *C. punctatus*. The estimated safe levels of hexaconazole in *C. punctatus*, as calculated by multiplying the LC_{50} with Application Factor (AF) recommended by different methods varied from 1.0327 to 10.327-1.0327 x 10^{-3}. However, the estimated safe levels cannot be guaranteed because of large variations found indifferent methods has resulted in controversy over its acceptability. In this approach, some safe limits in extrapolation of laboratory experiments to field are not always meaningful value and hence, it is difficult to decide an acceptable concentration based on the laboratory experiments that may be

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considered 'safe' in the environmental concentrations. Kennega emphasized that the major weakness in calculation of Accumulation Factor (AF) is its dependence on LC_{50} value.

Acute toxicity data has been used to derive water quality guidelines for regulatory assessment. Physiological parameters like quality, temperature, pH, dissolved oxygen and turbidity of water, amount and kind of aquatic vegetation, concentration and formulation of chemical and its exposure also greatly influence such studies. Toxicities of chemicals to aquatic fauna has been shown to be affected by age, size health and experimental factors of the species.

The behavioural study gives direct responses of the fish to the pesticides and related chemicals. Radhaiah et al., and Warner et al., commented that the behavioral activities of an organism represent the final integrated results of a diversified biochemical and physiological processes. The fish from the control group were free from any such type of behavioral changes. So it is clear that only hexaconazole was responsible for the altered behaviour and mortality. Similar results on behavioral responses were also observed in pesticide, heavy metals, and industrial effluents on exposed fish. Animal behaviour is a neurotropically regulated phenomenon, which is mediated by neurotransmitter substances. The preceding behavioural abnormalities of the fish and subsequent death imply that the toxic effect is mediated through the disturbed nervous/cellular enzyme system affecting the respiratory function and nervous system, which involves control of almost all vital activities.

The present study demonstrated that fish are highly sensitive to hexaconazole and that their mortality rate is dose dependent. This study also shows that fish are highly sensitive to hexaconazole and that their mortality rate is dose dependent and the significance of behavioral parameter in assessing the hazards of the pesticide to fish.

CONCLUSION

From the above investigation, we conclude that fungicide hexaconazole is highly toxic against freshwater fish *C. punctatus*. Moreover, fungicides used on farmlands not only eradicate the target organisms, but could also eliminate other nontarget organisms and also pose a risk to human health. It should be used carefully for agricultural and industrial purposes, and technologies must be adopted standards of safety for protection of nontarget organisms and environments.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Farah MA, et al. Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. Chemosphere. 2004;55:257-265.
2. Bansal SK, et al. The effects of some divalent metals on cardiac and branchial Ca^{2+}-ATPase in a freshwater fish *Saccobranchus fossilis*. Ecotoxicol Environ Saf. 1985;9:373-377.
3. Bhatnagar MC, et al. Respiratory distress to *Clarias batrachus* (Linn.) exposed to endosulfan-a histological approach. J Environ Biol. 1992;13:227–231.
4. Bony S, et al. Genotoxic pressure of vineyard pesticides in fish: Field and mesocosm surveys. Aquat Toxicol. 2008;89:197-203.
5. Çavaş T, et al. Micronucleus test in fish cells: A bioassay for in situ monitoring of genotoxic pollution in the marine environment. Environ Mol Mutagen. 2005;46:64-70.
6. Galloway T, et al. Immunotoxicity of organophosphorous pesticides. Ecotoxicology. 2003;12:345-363.
7. Ganeshwade RM, et al. Behavioral responses of *Cyprinus carpio* to industrial effluents. J Environ Biol. 2006;27:159.
8. Gupta PK, et al. The temperature dependence of the acute toxicity of copper to a freshwater pond snail, *Viviparus bengalensis* L. Hydrobiologia. 1981;83:461-464.
9. Han J, et al. Bioactivity, toxicity and dissipation of hexaconazole enantiomers. Chemosphere. 2013;93:2523-2527.
10. Hernández AF, et al. Toxic effects of pesticide mixtures at a molecular level: their relevance to human health. Toxicology. 2013;307:136-145.
11. IJC. New and revised great lakes water quality objectives. Great Lake Basin, Windsor. Ottawa, Canada. 1977.
12. Janaki Devi V, et al. Genotoxic effects of profenofos on the marine fish, *Therapon jarbua*. Toxicol Mech Methods. 2012;22:111-117.
13. Jha AN, et al. Ecotoxicological applications and significance of the comet assay. Mutagenesis. 2008;23:207-221.

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p-ISSN: 2347-2294

14. Khalili M, et al. Acute toxicity test of two pesticides diazinon and deltamethrin, on swordtail fish (*Xiphophorus helleri*). Glob Vet. 2012;8:541-545.
15. Kumar PR. et al. Alterations in gonado somatic index, seminogram and testosterone profiles in rats treated with hexaconazole. Res Opin Anim Vet Sci. 2011;1:78-82.
16. Kumar R, et al. Investigation of the genotoxicity of malathion to freshwater teleost fish *Channa punctatus* (Bloch) using the micronucleus test and comet assay. Arch Environ Contam Toxicol. 2010;58:123-130.
17. Larkin DJ, et al. Fate and effects of diazinon. Rev Environ Contam Toxicol. 2000;166:49-82.
18. Li Y, et al. Studies of enantiomeric degradation of the triazole fungicide hexaconazole in tomato, cucumber, and field soil by chiral liquid chromatography–tandem mass spectrometry. Chirality. 2013;25:160-169.
19. Manikandan SR, et al. Hematological studies on freshwater catfish *Mystus vittatus* exposed to sodium arsenate. Int J Pure Appl Zool. 2013;1:310-314.
20. Mishra A, et al. Acute toxicity and behavioral response of freshwater fish, *Mystus vittatus* exposed to pulp mill effluent. J Environ Chem Ecotoxicol. 2011;3:167-172.