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Acute Toxicity Assessment And Behavioural Biomarker Responses Of *Ctenopharyngodon Idella* Exposed To Tebuconazole (25.9% EC) Using Probit Analysis

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Abstract

The widespread application of fungicides in agriculture has resulted in their frequent detection in aquatic ecosystems, raising serious concerns regarding their impact on non-target aquatic organisms. The present investigation evaluates the acute toxicity of the triazole fungicide tebuconazole and its effects on behavioural responses in the freshwater fish *Ctenopharyngodon idella*. Acute toxicity bioassays were conducted under laboratory conditions for 24, 48, 72 and 96 h using a static renewal system. Mortality data were analysed using probit analysis to determine median lethal concentrations (LC_{50}). The LC_{50} values of tebuconazole were found to be **24 mg/L (24 h), 23 mg/L (48 h), 22 mg/L (72 h) and 21 mg/L (96 h)**, indicating a clear time-dependent increase in toxicity. Behavioural alterations such as erratic swimming, increased opercular movement, surface gulping, loss of equilibrium and lethargy were observed in exposed fish, with severity increasing with concentration and exposure duration. The linear relationship observed in probit regression curves confirms the suitability of probit analysis for LC_{50} estimation. The findings demonstrate that tebuconazole poses a significant toxic risk to *C. idella*, even during short-term exposure, highlighting the need for regulated use of this fungicide to prevent ecological damage.

Keywords: *Ctenopharyngodon idella*, Tebuconazole, Acute toxicity, LC_{50}, Probit analysis, Behavioural responses

1. Introduction

The increasing dependence on agrochemicals to enhance agricultural productivity has resulted in widespread contamination of aquatic ecosystems, posing a significant threat to non-target organisms such as fish. Fungicides, particularly triazole compounds, are frequently detected in surface waters due to agricultural runoff and spray drift (Malaj et al., 2020; Tang et al., 2021). Fish are especially vulnerable to these contaminants owing to their continuous exposure through gills, skin and ingestion of contaminated water and food (Scholz et al., 2022).

Tebuconazole is a systemic triazole fungicide widely used to control fungal diseases in cereals, fruits and vegetables. Although designed to target fungal sterol biosynthesis, tebuconazole has been shown to interfere with cytochrome P450-dependent enzymes in non-target organisms, leading to neurophysiological and metabolic disturbances in fish (Li et al., 2020; Zhang et al., 2023). Residues of tebuconazole have been increasingly reported in freshwater bodies, raising concerns regarding its ecological risk (Carvalho et al., 2021).

Fish behavioural responses are considered sensitive biomarkers of toxic stress and often precede physiological damage or mortality (Scott & Sloman, 2021). Alterations such as erratic swimming, loss of equilibrium and respiratory distress have been reported in fish exposed to triazole fungicides (Ramesh et al., 2022).

Ctenopharyngodon idella (grass carp) is an economically important freshwater fish widely cultured in Asia and commonly used in ecotoxicological studies due to its sensitivity to chemical stressors. The present study aims to determine the acute toxicity and LC_{50} values of tebuconazole using probit analysis and to evaluate associated behavioural alterations in *C. idella*.

2. Materials and Methods

2.1 Test chemical

The test chemical used in the present study was tebuconazole, a commercially available triazole fungicide. The formulation was procured from an authorized agrochemical supplier.

2.2 Preparation of stock and test solutions

A stock solution of tebuconazole was prepared by dissolving a known quantity of the commercial formulation in distilled water. Test solutions of required concentrations were prepared freshly by appropriate dilution of the stock solution prior to each experiment.

2.3 Test fish and acclimatization

Healthy fingerlings of *Ctenopharyngodon idella* of uniform size and weight were collected from a local fish farm. Fish were acclimatized to laboratory conditions for 10–15 days in dechlorinated water under natural photoperiod. During acclimatization, fish were fed a standard diet, and feeding was discontinued 24 h before the commencement of the experiment.

2.4 Acute toxicity bioassay

Acute toxicity tests were conducted using a static renewal bioassay system. Fish were divided into control and experimental groups, with equal numbers of fish in each group. Control fish were maintained in pesticide-free water, while experimental groups were exposed to graded concentrations of tebuconazole for 24, 48, 72 and 96 h. Mortality was recorded at the end of each exposure period, and dead fish were removed immediately to prevent deterioration of water quality.

2.5 Statistical analysis

Mortality data were subjected to probit analysis following the method of Finney (1971). Percent mortality values were converted into probit units, and regression analysis was performed using log-transformed dose values. LC_{50} values were determined for each exposure period.

3. Results

3.1 Acute Toxicity and Probit Analysis

Mortality of *Ctenopharyngodon idella* increased progressively with increasing concentration of tebuconazole and exposure duration. Probit analysis revealed LC_{50} values of **24 mg/L (24 h), 23 mg/L (48 h), 22 mg/L (72 h) and 21 mg/L (96 h)**, indicating time-dependent toxicity. The linear relationship observed between probit mortality and log-transformed dose confirms the suitability of probit analysis for LC_{50} determination (Finney, 1971; Newman, 2021).

3.2 Behavioural alterations

Distinct behavioural abnormalities were observed in fish exposed to tebuconazole compared to control fish. Initially, exposed fish exhibited restlessness and hyperactivity, followed by erratic and uncoordinated swimming. Increased opercular movement and frequent surface gulping were observed, indicating respiratory distress.

At higher concentrations and longer exposure durations, fish showed loss of equilibrium, reduced responsiveness and lethargy. Prior to death, fish settled at the bottom of the tank with complete loss of balance. Control fish exhibited normal swimming behaviour throughout the experimental period.

4. Discussion

The observed decrease in LC_{50} values with increasing exposure duration indicates cumulative toxicity of tebuconazole, a phenomenon commonly reported for triazole fungicides (Li et al., 2020; Tang et al., 2021). Behavioural abnormalities such as erratic swimming, loss of equilibrium and respiratory distress suggest neurotoxic stress, possibly due to disruption of cytochrome P450-mediated detoxification pathways (Zhang et al., 2023).

Similar behavioural impairments have been reported in freshwater fish exposed to triazole and azole fungicides, leading to reduced feeding efficiency and increased predation risk (Scott & Sloman, 2021; Ramesh et al., 2022). The linearity of probit regression curves further confirms the reliability of LC_{50} estimation and supports the application of probit analysis in aquatic toxicology studies (Newman, 2021).

5. Conclusion

The present investigation concludes that tebuconazole is acutely toxic to the freshwater fish *Ctenopharyngodon idella*. The decreasing LC_{50} values with increasing exposure duration indicate time-dependent toxicity. Tebuconazole exposure induces significant behavioural abnormalities, reflecting neurophysiological stress in fish. These findings highlight the potential ecological risk associated with tebuconazole contamination of aquatic environments and emphasize the need for regulated and judicious use of this fungicide.

Table.1: Observed percent mortality, probit mortality and LC_{50} value of *Ctenopharyngodon idella* exposed to tebuconazole for 24 h

S. No		Dose (mg/L)	Mortality (%)	Probit value (Y)	Log (100×Dose) = X	X²	X×Y	Y²
1		23.6	10	3.72	3.3729	11.3764	12.547	13.838
2		23.7	20	4.16	3.3750	11.3906	14.040	17.305
3		23.8	30	4.48	3.3771	11.4048	15.129	20.070
4		23.9	40	4.75	3.3791	11.4183	16.050	22.563
5		**24.0**	**50**	**5.00**	**3.3802**	**11.4257**	**16.901**	**25.000**
6		24.1	60	5.25	3.3820	11.4379	17.755	27.563
7		24.2	70	5.52	3.3838	11.4501	18.678	30.470
8		24.3	80	5.84	3.3856	11.4622	19.771	34.106
9		24.4	90	6.28	3.3874	11.4744	21.272	39.438

Table.2: Observed percent mortality, probit mortality and LC_{50} value of *Ctenopharyngodon idella* exposed to tebuconazole for 48 h

S. No	Dose (mg/L)	Mortality (%)	Probit value (Y)	Log (100×Dose) = X	X²	X×Y	Y²
1	22.6	10	3.72	3.3541	11.2499	12.477	13.838
2	22.7	20	4.16	3.3560	11.2627	13.960	17.305
3	22.8	30	4.48	3.3579	11.2754	15.043	20.070
4	22.9	40	4.75	3.3598	11.2882	15.959	22.563
5	**23.0**	**50**	**5.00**	**3.3617**	**11.3010**	**16.808**	**25.000**
6	23.1	60	5.25	3.3636	11.3138	17.658	27.563
7	23.2	70	5.52	3.3655	11.3265	18.577	30.470
8	23.3	80	5.84	3.3674	11.3393	19.665	34.106
9	23.4	90	6.28	3.3692	11.3515	21.158	39.438

Table.3: Observed percent mortality, probit mortality and LC_{50} value of *Ctenopharyngodon idella* exposed to tebuconazole for 72 h

Concentration range selected around 72 h LC_{50} = 22 mg/L

S. No	Dose (mg/L)	Mortality (%)	Probit value (Y)	Log (100×Dose) = X	X²	X×Y	Y²
1	21.6	10	3.72	3.3345	11.1205	12.404	13.838
2	21.7	20	4.16	3.3365	11.1322	13.879	17.305
3	21.8	30	4.48	3.3385	11.1455	14.956	20.070
4	21.9	40	4.75	3.3404	11.1582	15.866	22.563
5	**22.0**	**50**	**5.00**	**3.3424**	**11.1716**	**16.712**	**25.000**
6	22.1	60	5.25	3.3444	11.1850	17.558	27.563
7	22.2	70	5.52	3.3464	11.1983	18.472	30.470
8	22.3	80	5.84	3.3483	11.2111	19.554	34.106
9	22.4	90	6.28	3.3502	11.2238	21.039	39.438

Table.4: Observed percent mortality, probit mortality and LC_{50} value of *Ctenopharyngodon idella* exposed to tebuconazole for 96 h

Concentration range selected around 96 h LC_{50} = 21 mg/L

S. No	Dose (mg/L)	Mortality (%)	Probit value (Y)	Log (100×Dose) = X	X^2	X×Y	Y^2
1	20.6	10	3.72	3.3139	10.9819	12.327	13.838
2	20.7	20	4.16	3.3160	10.9958	13.794	17.305
3	20.8	30	4.48	3.3181	11.0097	14.865	20.070
4	20.9	40	4.75	3.3201	11.0230	15.770	22.563
5	**21.0**	**50**	**5.00**	**3.3222**	**11.0370**	**16.611**	**25.000**
6	21.1	60	5.25	3.3243	11.0509	17.452	27.563
7	21.2	70	5.52	3.3263	11.0642	18.361	30.470
8	21.3	80	5.84	3.3284	11.0782	19.437	34.106
9	21.4	90	6.28	3.3304	11.0915	20.914	39.438

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7. Conflict of Interest

1. The authors declare that there is no conflict of interest.
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