Toxicity of tricyclazole on certain serum biochemical markers of an Indian paddy-field fish, Channa punctatus (Bloch)

Dina Nath Pandit and Usha Rani

Abstract
This maiden study was designed to evaluate acute toxicity of tricyclazole and its responses on certain biomarker in a freshwater fish Channa punctatus (Bloch). By Reed-Muench method, mean 96hr-LC50 dose of tricyclazole for this fish was calculated 25.0 mg l⁻¹. The LC50 dose indicates highly toxic nature of tricyclazole. Fish were then treated to 0.25 and 1.25 mg l⁻¹ sublethal dose of tricyclazole for short term long term exposure and the alterations in activities of glucose, protein, urea and cholesterol were determined in serum. Levels of glucose, urea and cholesterol in fish exposed to tricyclazole increased significantly (p<0.05) at all durations of short-term and long-term exposure. Conversely, protein decreased significantly (p<0.05) in response to tricyclazole at both the concentration and all exposure periods. Therefore, exposure to tricyclazole at sub-lethal concentrations induces severe serum biochemical alterations in Channa punctatus that may potentially disrupt their survival in their natural habitat. These findings may be used in the assessment of the potential risk of tricyclazole on food chain and aquatic ecosystems.

Keywords: Tricyclazole, Channa punctatus, toxicity, biochemical markers

1. Introduction
Toxicity tests are designed to predict concentration of a toxicant and its duration of treatment that produce an undesirable effect. Acute toxicity test helps in identification of mode of action of a toxicant and information on dose associated with mortality that can be used to set dose levels for repeated-dose studies. Reed & Muench (1938) [21] method is generally opted by researchers in Experimental Biology because of its ease of application.

Fungicides (e.g. tricyclazole and propiconazole) are used to increase yield of crop finally find their way into the aquatic ecosystem. Tricyclazole is one of the fungicides recommended to treat of blast diseases caused by Pyricularia oryzae in paddy. Tricyclazole is highly toxic substance and has a high risk of environmental contamination (Loomis & Hayes 1996, Padovani et al 2006, Sancho et al 2009 and Naik et al 2012) [10, 17, 25, 14]. The use of tricyclazole in paddy culture leads to harmful effects on various parameters of aquatic non-target species. Effect of propiconazole on biochemical profile of this fish is studied (Tabassum et al 2018) [29].

Channa punctatus (Bloch), a freshwater Indian air breathing fish, is found in paddy fields. However, the toxicity of a fungicide on Indian freshwater fish is lacking in this context. Therefore, the present work was designed to evaluate LC50 of tricyclazole and alterations in certain biochemical parameters in intoxicated Channa punctatus. The study will be helpful to evaluate variation in health condition of fungicide exposed fish, suitability of environmental conditions for fish and relative sensitivity of fish to tricyclazole.

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 from 2018 to 2019. The fishes were disinfected with dilute KMnO4 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. Treatments
The experiments were conducted every time in between 11 AM to 1 PM. The physico-chemical features of experimental water were determined following standard method (APHA 2012) [2]. Tricyclazole (75% EC; Molecular formula: C_{18}H_{20}N_{5}S; Molecular mass: 189.24g/mol) is a fungicide manufactured by Bayer Crop Science Ltd., Gujarat, India. In each selected concentration of Tricyclazole, ten fishes from stock were kept. With the help of the records of dead fishes, 96h-LC50 doses were determined by Reed & Muench (1938) [21]. From this value, 1% and 5% of 96h-LC50 dose was used for serum biochemical studies.

2.3. Observations
The blood samples were collected by heart puncture method. After coagulation, the uncoagulated and coagulated part were separated and centrifuged at 3000 rpm for 10 minutes. Serum glucose, protein, urea and cholesterol of separated and centrifuged at 3000 rpm blood samples were used for serum biochemical studies.

2.4. Data Analysis
Data analysis was performed with the use of Graph Pad Prism 5. Results were presented as mean and standard deviation. The differences between the control and treated groups were compared by using t-test and F-test (p<0.05).

3. Results
The temperature 26.0±2.0 °C, pH: 7.14±0.08, dissolved oxygen: 6.4±0.4 mg l−1, total alkalinity: 56.0±4.5 mg l−1, hardness: 150.6±5.2 mg l−1 and chloride: 16.7±0.2 mg l−1 of experimental water was recorded to which fish were exposed. The data of LC50 of tricyclazole on Channa punctatus (Bloch) have been summarized in Table 1. The lethal range of tricyclazole from 0.01, 0.1, 1.0, 10.0 and 100.0 mg l−1 following Reish & Oshida (1987) [23] method was found. Mortality was lacking in 0.01 to 10.0 mg l−1 while total mortalities were found in 100.0 mg l−1 of tricyclazole. From Reed & Muench (1938) [21] method, 96hr-LC50 dose of 24.0 and 25.02 mg l−1 as MLC50 and MSC50 was calculated. The concentration of 96hr-LC50 was done applying following cross checks:

(a) The ideal LC50 was calculated from the mean of MLC50 and MSC50

\[
LC_{50} = \frac{MLC_{50} + MSC_{50}}{2} = 24.51\ mg\ l^{-1}.
\]

(b) Sum of doses and sum of mortalities: Its value was

\[
\frac{MLC_{50} + MSC_{50}}{2} = 24.51\ mg\ l^{-1}.
\]

When 5.26 were multiplied with 5 gave the product as 26.30. Therefore, LC50 = 26.30 mg l−1 and (c) The average 96hr-LC50 dose of tricyclazole from this method was calculated to be 24.0±25.02±26.30= 25.107 or 25.0 mg l−1 (Table 1).

Mean glucose of 79.1±3.64 mg dl−1 of controlled fish was found to increase significantly (p<0.05) from 87.50±1.72 to 127.96±12.9 mg dl−1 during short-term while from 86.93±1.89 to 125.86±2.14 mg dl−1 during long-term treatment of 0.25 and 1.25 mg l−1 tricyclazole. The hyperglycemia shows more dependency on duration compared to the dose of tricyclazole (Table 2). The elevation in glucose was more pronounced in short term treatment compared to long term exposure. Conversely, mean protein amount of 4.97±0.11 g dl−1 in control fish decreased significantly (p<0.05) from 4.52±0.10 to 3.40±0.80 g dl−1 during short-term and from 4.48±0.10 to 3.29±0.06 g dl−1 during long-term treatment of 0.25 and 1.25 mg l−1 tricyclazole respectively. The fall in protein was less in short term compared to long term exposure. The decrease shows equal dependency on both duration and dose of tricyclazole (Table 3).

Mean area of 15.0±0.37 mg dl−1 of control fish increased significantly (p<0.05) from 17.19±0.34 to 23.39±0.19 mg dl−1 during short-term and from 16.20±0.12 to 24.87±0.15 mg dl−1 during long-term treatment of 0.25 and 1.25 mg l−1 tricyclazole respectively. Hyperuremia was found more in long term treatment in comparison to short term exposure. Hyperuremia shows much more dependency on duration than the dose of tricyclazole (Table 4).

Similarly, mean cholesterol level of 80.0±4.0 mg dl−1 in control fish increased significantly (p<0.05) from 100.5±2.27 to 133.69±1.92 mg dl−1 during short-term and from 98.39±1.25 to 131.68±2.11 mg dl−1 during long-term treatment of 0.25 and 1.25 mg l−1 tricyclazole respectively. The hypercholesterolemia was more in long term treatment compared to short term exposure. The hypercholesterolemia shows much more dependency on duration than the dose of tricyclazole (Table 5).

### Table 1: Reid-Muench (1938) method for 96hr-LC50 determination of tricyclazole in Channa punctatus

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Dose (mg l−1)</th>
<th>Log dose (mg l−1)</th>
<th>Experiment</th>
<th>Specific Cumulative</th>
<th>Rate of mortality</th>
<th>% mortality</th>
<th>% survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No of mortality</td>
<td>No. of survival</td>
<td>Mortality</td>
<td>Survival</td>
<td>Total</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>1.000</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>36</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>1.301</td>
<td>3</td>
<td>7</td>
<td>6</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>1.477</td>
<td>6</td>
<td>4</td>
<td>12</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>1.602</td>
<td>9</td>
<td>1</td>
<td>21</td>
<td>1</td>
<td>22</td>
</tr>
</tbody>
</table>

### Calculation of median lethal concentration (LC50)

\[
MLC_{50} = \frac{258.29 + 204.10}{2} = 24.51\ mg\ l^{-1}.
\]

### Calculation of median survival concentration (MSC50)

\[
MLC_{50} = \frac{55.86 - 28.41}{2} = 25.02\ mg\ l^{-1}.
\]
Table 2: Variations in serum glucose (mg dl⁻¹) of *Channa punctatus* after acute and chronic exposure to two sub-lethal dose of tricyclazole

<table>
<thead>
<tr>
<th>Sub-lethal Dose of tricyclazole (mg l⁻¹)</th>
<th>Normal range of glucose in fish</th>
<th>Controlled value of glucose</th>
<th>Duration of exposure</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>a=24 hours b=15 days</td>
<td>a=48 hours b=30 days</td>
</tr>
<tr>
<td>0.25</td>
<td>20.00-100.00</td>
<td>79.13±3.64</td>
<td>87.50±1.72 (+10.58%)*</td>
<td>102.76±2.96 (+29.86%)**</td>
</tr>
<tr>
<td>1.25</td>
<td></td>
<td></td>
<td>86.93±1.89 (+9.88%)*</td>
<td>100.29±2.03 (+26.74%)**</td>
</tr>
<tr>
<td>Average</td>
<td>90.78±3.74 (+12.91%)*</td>
<td></td>
<td>110.64±9.19 (+39.82%)*</td>
<td>115.13±11.82 (+45.49%)*</td>
</tr>
</tbody>
</table>

(*= Significant, **= Moderately Significant, ***= Highly Significant)

Table 3: Variations in serum total protein (g dl⁻¹) of *Channa punctatus* after acute and chronic exposure to two sub-lethal dose of tricyclazole

<table>
<thead>
<tr>
<th>Sub-lethal Dose of tricyclazole (mg l⁻¹)</th>
<th>Normal range of total protein in fish</th>
<th>Controlled value of total protein</th>
<th>Duration of exposure</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>a=24 hours b=15 days</td>
<td>a=48 hours b=30 days</td>
</tr>
<tr>
<td>0.25</td>
<td>3.00-10.00</td>
<td>4.97±0.11</td>
<td>4.52±0.10 (-9.05%)*</td>
<td>4.48±0.12 (-9.86%)*</td>
</tr>
<tr>
<td>1.25</td>
<td></td>
<td></td>
<td>4.48±0.10 (-9.86%)*</td>
<td>4.40±0.10 (-11.47%)*</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>4.44±0.08 (-10.66%)*</td>
<td>4.38±0.09 (-11.87%)*</td>
</tr>
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Table 4: Variations in serum urea (mg dl⁻¹) of *Channa punctatus* after acute and chronic exposure to two sub lethal dose of tricyclazole

<table>
<thead>
<tr>
<th>Sub-lethal Dose of tricyclazole (mg l⁻¹)</th>
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<th>Controlled value of urea</th>
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<th>F value</th>
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<td></td>
<td></td>
<td>a=24 hours b=15 days</td>
<td>a=48 hours b=30 days</td>
</tr>
<tr>
<td>0.25</td>
<td>2.50 -20.00</td>
<td>15.0±0.37</td>
<td>17.41±0.84 (+16.07%)*</td>
<td>19.39±0.65 (+35.93%)**</td>
</tr>
<tr>
<td>1.25</td>
<td></td>
<td></td>
<td>16.20±0.12 (+8.00%)*</td>
<td>17.10±0.24 (+14.0%)*</td>
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<tr>
<td>Average</td>
<td></td>
<td></td>
<td>17.19±0.34 (+18.60%)*</td>
<td>21.67±0.78 (+44.47%)*</td>
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<td></td>
<td></td>
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<td>22.59±0.19 (+50.60%)*</td>
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4. Discussion

The toxicity determination of fungicides seems essential to evaluate the sensitivity and damage to specific organs/systems of an animal (Singh et al 2010) [28]. Such observations results as outcome of various diseases in fishes and also reveals underlying physiological conditions of the organs and tissues (Obomanu et al 2009) [15].

For this study, experimentation with 10 fishes per dose is necessary for better correlation. The method was modified by Sagananu (2011) [24] in calculating survival and mortality of percent of test animals to arrive a conclusion. Although, 95% confidence limit cannot be calculated with this method. 96h-LC₅₀ dose of tricyclazole in *Channa punctatus* rates it as a most toxic substance according to toxicant classification (Loomis & Hayes 1996) [10].

The hyperglycemia indicates disrupted carbohydrate metabolism probably after the elevated glycosylation. Ferrando & Moliner (1991) [6] saw hyperglycemia in pesticide intoxicated *Channa punctatus*, *Heteropneus fossilis* and *Cyprinus carpio*. Hyperglycemia was also reported by Firtet al (2011) [27] in *Oreochromis niloticus* exposed to copper, lead and cypermethrin. Borges et al (2007) [4] suggested that cypermethrin induced hyperglycemia in *Rhodina quelen* as a sign of stress. The hyperglycemia is a reliable indicator of environmental stress due to general secondary response of intoxicated fish (Sepici-Dinkel et al 2009) [26]. Hyperglycemia seems to be mediated through ACTH and reduced insulin secretion, increased corticosteroid and glycocongenes (Ramesh & Saravana 1985) [20]. The present finding is in close consortium with the above observations.

The decrease in protein is supported by the findings of Velisek et al (2007) [30] who observed its significant decrease in metribuzin treated rainbow trout. Min & Kang (2008) [11] additionally referred decline vague in *Nile tilapia* after benzoyl toxicity. A reduction in protein was also reported by Firt et al (2011) [7] in *Oreochromis niloticus* exposed to copper, lead and cypermethrin. Conversely, Shaikh et al (2014) [27] stated increase in protein of *Channa punctatus* after nuxan treatment. The decrease of protein may be due to the inhibition of transcription disturbing the protein metabolism or liver injury where most translation occurs (Reddy & Vinnela, 2016) [22]. Reduction in protein may also be attributed to intensive proteolysis which contributes after the increase within the free amino acids to be fed in Krebs’ cycle. These outcomes may agree with findings of Ram & Singh (1998) [19], when carbofuran triggered biochemical changes in
Channa punctatus treatment resulted in massive decrease in the protein content in various tissues/organisms of fish. Hyperuremia observed in this work was also reported by David et al. (2004) [5] in Cyprinus carpio and Kumar et al. (2011) [9] exposed to cypermethrin and Balasubramaniam & Kumar (2013) [3] in Heteropneustes fossilis induced to sodium arsenate. The air-breathing fish are ureogenic having extraordinary level of activity for enzymes of urea cycle. Channa punctatus exposed to different concentrations of carbaryl exhibit much biochemical alteration into urea and relates after renal failure in Cyprinus carpio (Lusкова et al 2002) [12]. The hypercholesterolemia following cypermethrin exposure was observed in Rhanda quelen (Borges et al 2007) [4]. An elevation in cholesterol was reported by Firat et al (2011) [7] in Oreochromis niloticus exposed to short- and long-term treatment of copper, lead and cypermethrin. Oner et al (2008) [16] found that cholesterol of metal-intoxicated Oreochromis niloticus increased compared to corresponding control value. They concluded that the concentration of cholesterol may increase due to hepatic and renal failure causing the release of cholesterol into the blood. Yousef et al (2003) [21] reported that changes in cholesterol are related to changes caused by pesticides in the permeability of hepatic cells and that accumulation of pesticides in the liver to disrupt lipid metabolism.

5. Conclusion
The range of serum glucose, protein, urea and cholesterol in control fish was within range of other fishes. Hyperglycemia, decreased serum protein, hyperuremia and hypercholesterolemia are indicators of altered carbohydrate, lipid and protein metabolism in the fish due to tricyclazole exposure. The changes are a reflection of organ dysfunction due to fungicide exposure. This study clearly indicates that the presence of tricyclazole even in small concentration may cause deleterious effects on fish physiology and may potentially disturb their survivability.

6. References


31. Yousef MI, El-Demerdash FM, Kamel KI Al-Salhen KS. Changes in some hematological and biochemical indices of rabbits induced by isoflavones and cypermethrin. Toxicol. 2003; 189:23-33