Study of acute toxicity of Metasystox on the freshwater fish, Nemacheilus botia, from Kedrai dam in Maharashtra, India

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Abstract
Acute toxicity of organophosphate pesticide Metasystox has been studied on freshwater fish Nemacheilus botia. The fishes have been used for many years to determine the pollution status of water. Static bioassays were performed on freshwater fish, N. botia to evaluate the median lethal concentrations of Metasystox (Oxydemeton-methyl) for 24, 48, 72 and 96 hrs. The LC₅₀ values were 10.3, 9.131, 7.884, and 7.018 ppm after 24, 48, 72 and 96 hours respectively. The results show that the LC₅₀ values decreased with increase in exposure period.

Keywords: Freshwater; Nemacheilus botia; toxicity; organophosphate pesticide; metasystox; LC₅₀.

Introduction
The widespread use of pesticides not only brought adverse influence on agro ecosystems but also caused alteration in physiological processes of non-target organisms. These pesticides through surface runoff reaches to the unrestricted areas like ponds and rivers which alters the physicochemical properties of water and is toxic to aquatic organism and cause deleterious effect or even death to the aquatic animals. In many countries, large scale mortality of fishes has been recorded due to pesticides in water bodies as pollutants.

The toxicity study is essential to find out toxicants limit and safe concentration, so that there will be minimum harm to aquatic fauna in the near future. Among the several aspects of toxicity studies, the bioassay constitutes one of the most commonly used methods in aquatic environmental studies with suitable organisms. The necessity of determining the toxicity of substances to commercially aquatic forms at the lower level of the food chain has been useful and accepted for water quality management. Several studies have been conducted in assessing the toxicity of pesticide to the aquatic biota especially fishes (Verma et al., 1982; Ravikrishnan et al., 1997; Vasit and Patil, 2005; Susan Anita et al., 2010). The wide use of fishes is probably due to their adaptability to the laboratory conditions as well as their availability.

Materials and Methods

and their varying degree of sensitivity to the toxic substance (Verma et al., 1980).

At present many organic pesticides like organophosphorus, organochlorine and methyl carbamates are currently used for agro-practices. During the last one decade a tremendous progress has been made in the development of new compounds with better toxicity, therefore, a lot of work has been carried out on impact of pesticides on non-target aquatic organisms (Battaglin and Fairchild, 2002; Prasanth et al., 2005). Organophosphates are highly toxic to fish and non-target aquatic organisms and are powerful nerve poisons, since they inhibit AChE activity (Coppage et al., 1975; Klaverkamp and Hobden, 1980). Several workers investigated the toxicity of organophosphorus pesticides in fish (Lockhart et al., 1973; Koundinya and Ramamurthi, 1979; Johnson and Finley, 1980; Kumar and Gupta, 1997; Santhakumar et al., 2000; Singh et al., 2010; Zhang et al., 2010; Srivastava et al., 2010; Barbieri and Ferreira, 2011; Maniyar et al., 2011). Perusal of literature reveals paucity of information on acute toxicity of metasystox on freshwater fish, Nemacheilus botia. Hence, the present study has been focused to evaluate the acute toxic effects of Metasystox on freshwater fish, N. botia, of local importance from Maharashtra state in Kedrai dam near Nashik.

The fish Nemacheilus botia were netted from Kedrai Dam, at Khadak Ozar near Chandwad
(Nashik) in the state of Maharashtra located on the coordinates of 20°, 17°, 30” N and 74°, 9°, 37” E. The fishes were brought to laboratory and release in glass aquaria (size 0.909 X 0.303 X 0.303 m.), where a continuous and gentle flow of tap water was maintained. The fishes were fed on fishmeal procured from market and allowed to acclimatize to laboratory conditions for one week. Water was aerated twice a day to prevent hypoxic conditions. Pilot experiments were conducted to find out the range of the toxicity of the toxicant used Metasystox. The chosen range of concentration was such that it resulted in 0 to 100% mortality. Stock solution (1 ppm) was prepared in tap water. The Series of statistic bioassay were conducted under laboratory condition as described by Finney (1964). Acute toxicity tests were conducted over 96 h. The experimental troughs containing 2 L dechlorinated water were used to keep the animals. For each experiment ten fishes, *N. botia* of approximately same weight ± 0.2 g and size 3 ± 1 cm were exposed to different concentrations of metasystox. After every 12 h the polluted water was changed by the fresh solution of the same concentration without any disturbance of fishes by static method. The mortality of the fishes was recorded before each change of water. The resulting mortality was noted in the range of 10 to 90% for each concentration for the duration of 24, 48, 72 and 96 h. Each experiment was repeated thrice to obtain constant results. The data collected was analyzed statistically by means of probit method on transforming toxicity curve (% mortality vs. concentration), which allows the average median lethal concentration of L50 to be calculated for 24, 48, 72 and 96 h. Dead fishes were counted individually.

**Results**

It was observed early in the experiment that the exposed fish exhibited altered behavior as compared to the controls. Immediately after adding the metasystox the fish became restless and started moving to and fro in the water. Then they started coming to the surface of the water to inhale air and it looked as through they were suffocating. Irregular, erratic and sometimes jerky movements were observed in fish exposed to metasystox. The opercular movement increased initially at moment of exposure time but after 2 h it became normal. They were easily frightened when tubs tapped. Within 24 h paralysis of body parts was observed in fishes kept in higher concentrations of metasystox. Muscles involved in movement of fins as well as the whole tail portion were seen constantly twitching before the fish died. The percent mortality of freshwater fish *N. botia* during the exposure of pesticide metasystox at different concentrations is shown in Table-1.

The LC50 values and exposure period showed a direct relationship. The LC50 values, regression equations, Chi square, variance and 95% fiducial limits, lethal concentration and safe concentration are shown in Table-2. The LC50 values obtained for metasystox exposed for 24, 48, 72 and 96 h exposure were 10.3, 9.131, 7.884, and 7.018 ppm for 24, 48, 72 and 96 h exposure respectively. From the above results it was observed that the LC50 values of 96 h were found lowest among all the exposure periods [Table-2].

Table 1: Percentage mortality of freshwater fish *N. botia* during the exposure of pesticide metasystox at different concentrations.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc. of pesticide (in ppm)</th>
<th>Percentage mortality at 24 h</th>
<th>Percentage mortality at 48 h</th>
<th>Percentage mortality at 72 h</th>
<th>Percentage mortality at 96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.125</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>7.25</td>
<td>-</td>
<td>10</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>7.875</td>
<td>-</td>
<td>20</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>8.25</td>
<td>10</td>
<td>30</td>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>8.625</td>
<td>10</td>
<td>40</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>10</td>
<td>50</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>7</td>
<td>9.375</td>
<td>30</td>
<td>50</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>9.75</td>
<td>40</td>
<td>60</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>10.13</td>
<td>40</td>
<td>70</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>10.5</td>
<td>50</td>
<td>80</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>10.88</td>
<td>70</td>
<td>90</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 2: Relative toxicity of pesticide metasystox against the freshwater fish *N. botia*.

<table>
<thead>
<tr>
<th>Time of exposure (Hrs.)</th>
<th>Regression equation ( Y = Y^* + (X - x)^* )</th>
<th>( L_{50} ) values in ppm</th>
<th>Variance ( V )</th>
<th>Chi-square</th>
<th>Fiducial limits M₁</th>
<th>Lethal dose</th>
<th>Safe conc. (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>( Y = 8.8828X - 21.9912 )</td>
<td>10.3</td>
<td>1.014 ( X^{10^2} )</td>
<td>1.2425</td>
<td>0.9935, 1.033</td>
<td>247.2</td>
<td>2.152</td>
</tr>
<tr>
<td>48</td>
<td>( Y = 13.6839X - 8.14426 )</td>
<td>9.131</td>
<td>1.014 ( X^{10^2} )</td>
<td>0.6854</td>
<td>0.9529, 0.968</td>
<td>438.29</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>( Y = 15.0228X - 8.4738 )</td>
<td>7.884</td>
<td>1.014 ( X^{10^2} )</td>
<td>1.7088</td>
<td>0.8687, 0.925</td>
<td>567.65</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>( Y = 10.8462X - 4.26750 )</td>
<td>7.018</td>
<td>1.014 ( X^{10^2} )</td>
<td>0.30875</td>
<td>0.6842, 1.008</td>
<td>673.73</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Fish mortality due to pesticide exposure mainly depends upon its sensitivity to the toxics, its concentration and duration of exposure. The evaluation of \( L_{50} \) concentration of pollutants is an important step before carrying out further studies on physiological changes in animals. In the present study the *N. botia* exposed to metasystox, the acute toxicity level was expressed in terms of \( L_{50} \) values. The \( L_{50} \) values were found to be 10.3, 9.131, 7.884, and 7.018 ppm at 24, 48, 72, and 96 h respectively. The percent survival rate of the fish decreased with increasing concentration and period of exposure. In present probe, acute toxicity test shows a relationship between the length of exposure period and concentration of pesticide. The \( L_{50} \) values of the fish decreases gradually as the exposure period goes on increasing. Acute toxicity involves the damage to the organism by fastest acting mechanism.

John (2007) investigated \( L_{50} \) value of Metasystox and Sevin to freshwater teleost, *Mystus vittatus* and \( L_{50} \) values were 6.5 ppm and 11.5 ppm for 30 days respectively. John et al. (1990) has reported \( L_{50} \) for Metasystox [demeton-S-methyl] after 96 h for the river fish *Heteropneustes fossilis* and *Ophiocephalus striatus* and \( L_{50} \) values were 12.9 ppm and 10.3 ppm respectively. The result of present study shows that \( L_{50} \) of Metasystox to *N. botia* for 96 h were lower than *H. fossilis* and *O. striatus* indicating that Metasystox is more toxic to *N. botia*. Pandir (1989) studied the acute toxicity levels of cadmium, lead, zinc and molybdenum to *Nemacheilus botia*.

Rashatwar (1981) evaluated \( L_{50} \) value of dimecron, BPMS (2-sec-butylphenyl methylcarbamate) and basalin in *Nemacheilus denisoni* and \( L_{50} \) values were 334.8 ppm for dimecron, 0.04571 ppm for BPMS and 0.005129 ppm for basalin for 96 h in *Nemacheilus denisoni*. Tilak and Kumari (2009) carried the acute toxicity of organophosphate Nuvan to the grass carp *Ctenopharyngodon idella* and \( L_{50} \) values were found to be 13.1, 10.9, 9.8 and 6.5 ppm at 24, 48, 72 and 96 h respectively.

Vasait and Patil (2005) investigated the \( L_{50} \) values of organophosphate pesticide to *N. botia* and calculated for 7 and 14 days exposure period. The result indicates decrease in \( L_{50} \) value with concentration and duration of exposure increase.

The effect of Metasystox on freshwater organisms is quite insufficient compared to marine organisms, so in the present work it is attempted to study the effect of Metasystox on survival of freshwater fish, *N. botia*.

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References


