EVALUATION OF THIODAN INDUCED CHANGES IN TOTAL PROTEIN AND ACETYLCHOLINESTERASE IN THE BRAIN OF OREOCHROMIS MOSSAMBICUS

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INTRODUCTION

Due to indiscriminate usage of pesticides in agriculture, there has been an immense disruption of the ecological balance causing damage to non target organisms including fish of commercial importance. Contamination of the environment by pesticides may cause several metabolic changes in fish (Yeragi et al., 2003). Fish have an important role in the food chain; therefore, investigation of the effects of pesticide on fish has a diagnostic significance in evaluation of adverse effects of pesticides to human health (Begum and Vijayaraghavan, 1996).

Teleostean fish are good indicators of aquatic contamination because their biochemical stress responses produced by a pollutant are similar to those found in mammals; it is therefore very easy to find similar explanations between both groups. The pollutants exert their effects at cellular/molecular or at organic/tissue level or on the whole organism. When these agents exceed the tolerance level to compensate the stress situation, death of the individual results. However, lethal effects are rare in nature because the organisms are exposed to low concentrations which are normally sublethal. The development of biochemical indicators which reflect these changes in aquatic organisms will allow a high precision in the detection of sublethal levels of pollutants released into the environment. These indicators can be developed as warning signals of contamination problems or to determine the health of the aquatic population (Haya et al., 1985). So the present study is to intend to evaluate the effects of the insecticide thiodan on the total protein and acetylcholinesterase in the brain of Oreochromis mossambicus in order to assess its possible toxicity.

MATERIALS AND METHODS

The commercial grade endosulfan (35% EC)-thiodan, supplied by Bayer crop science India Ltd., Mumbai was used. The required concentration of the pesticide was prepared in chlorine free tap water using acetone as solvent. Fresh water acclimated fish having a size of 8-10 cm and 10-12 g wet weight were used. The maintenance and acclimation of fish was done using standard procedures as described earlier (Abhilash and Prakasam, 2005). The 96 h LC50 value calculated using probit analysis with SPSS/Windows (SPSS.10.0.LNK) was found to be 1.4mg/L. The fish were grouped into four, each consisting of ten individuals. First group served as control and the remaining as experimental. The fish were exposed to different sub lethal concentrations (1/20th, 1/15th and 1/10th LC50) of the toxicant. The fish were fed with standard food pellets throughout the experiment, but feeding was stopped 24 h prior to each sampling day. Controls received similar treatment except the addition of thiodan. Samples were collected at the end of 10th, 20th and 30th days of exposure. The estimation of total protein and acetylcholinesterase

ABSTRACT

The effect of thiodan, a commercial grade endosulfan on total protein and acetylcholinesterase in the brain of Oreochromis mossambicus was studied at sublethal concentrations of 1/20th, 1/15th and 1/10th of LC50 value and at 10, 20 and 30 days of exposure. The brain protein content got diminished significantly in response to increase in time and dose of thiodan. The highest decrease was observed on the 30th day and at 1/10th LC50 concentration. Increased proteolysis due to the stress posed by thiodan was evident. Acetylcholinesterase activity in the brain of exposed fish was found inhibited at all sublethal concentrations demonstrating the neurotoxic effect of the insecticide on the fish. Thus the present study indicated the toxic perturbations in the metabolic profiles of the fish exposed to Thiodan.

KEY WORDS
Thiodan
Oreochromis mossambicus
Brain
Acetylcholinesterase
Total protein

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RESULTS

Table 1 shows that a duration dependent reduction in brain protein content was recorded in the exposed fish at all the three sublethal concentrations on the 10th, 20th and 30th day respectively. Of the three sublethal concentrations, the effect was most prominent at the highest sublethal concentration \( \dfrac{\text{LC}_{50}}{10} \). It can be seen that fish exposed to the highest sublethal concentration on the 10th day recorded 29.80% reduction in protein content than the control value. It was further reduced to 44.17% on the 20th day and to 61.04% on the 30th day when compared to control. Thus in the case of brain, the protein content got diminished significantly in response to thiodan. As seen from Table 2, a dose and time dependant diminution in AChE activity level was recorded in the brain of \( O. \ mossambicus \). A more significant duration dependent diminution in AChE activity was registered at the intermediate sublethal concentration. The fish exposed to the highest sub lethal concentration also exhibited the same trend but, the decrease in percentage level over control was more pronounced here. Thus, the highest sub lethal concentration on the 30th day caused the most pronounced decrease in AChE activity.

DISCUSSION

The protein metabolism of fish is known to be altered due to the stress posed by various contaminants, especially pesticides. Even protein depletion in the brain of fish, subjected to various pesticides, has been reported. Reddy and Bashamohideen (1995) recorded decline in brain protein content in the fish \( Cyprinus \ carpio \) exposed to cypermethrin. Later, Rita and Milton (2006) while studying the effect of the carbamate pesticide lannate recorded depletion in protein content in the brain of the fish \( O. mossambicus \). In the present investigation also protein loss was registered in the test organism subjected to thiodan. It was to the extent of 7.87% to 61.04%. This might be due to excessive proteolysis to overcome the metabolic stress caused by the insecticide, as opined by Naveed et al. (2010). But such large scale protein depletion in tissues of vital importance like brain might impair the biochemical, physiological and behavioural well-being of the fish and ultimately threaten its survival. Acetylcholinesterase is an important enzyme of the hydrolase group that facilitates the conduction of nerve impulses across a synapse. It is also involved in the maintenance of the structural and functional integrity of cellular membrane (De Robertis et al., 1975). Studies on the level of tissue AChE activity have been conventionally undertaken to secure information on the mode of toxic action of different pesticides. Brain AChE activity was reportedly seen inhibited when \( Cyprinus \ carpio \) was exposed to chloropyrifos (Halappa and David (2009). Organophosphate and carbamate pesticides are specifically known for their anticholinesterase activity. But some organochlorines would also act as nerve poisons (Baskaran and Pandian, 1987). In the present study a time and dose dependent diminution in AChE activity was noted in \( O. mossambicus \) exposed to different sub lethal concentrations of thiodan. A duration dependent slight reduction in AChE activity was measured at the lowest sub lethal concentration. A more significant duration dependent diminution in AChE activity was registered at the intermediate sublethal concentration. The fish exposed to the highest sub lethal concentration also exhibited the same trend but, the decrease in percentage level over control was more pronounced here. Thus, the highest sub lethal concentration on the 30th day caused the most pronounced decrease in AChE activity.

CONCLUSION

An important observation made in the study was the depletion of protein and inhibition of AChE activity in the brain tissue of the exposed fish. The depletion of protein fraction in brain tissue might be due to their degradation and possible utilization for metabolic purposes. AChE inhibition could lead to increased accumulation of acetylcholine, which in turn induce behaviour abnormalities like restlessness and tremor. The fall

Table 1: Total protein content (mg/g) in the brain of control \( O. mossambicus \) and that exposed to sublethal concentrations of Thiodan

<table>
<thead>
<tr>
<th>Days</th>
<th>Concentration</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39.36 ± 1.14</td>
<td>40.50 ± 1.24</td>
<td>42.33 ± 1.08</td>
<td></td>
</tr>
<tr>
<td>( \dfrac{\text{LC}_{50}}{10} )</td>
<td>36.26 ± 1.74 (∆7.87)</td>
<td>34.47 ± 1.48 (∆14.88)</td>
<td>30.38 ± 1.44 (∆39.40)</td>
<td></td>
</tr>
<tr>
<td>( \dfrac{\text{LC}_{10}}{10} )</td>
<td>32.27 ± 2.14 (∆18.01)</td>
<td>29.26 ± 1.86 (∆27.75)</td>
<td>25.65 ± 2.16 (∆39.40)</td>
<td></td>
</tr>
<tr>
<td>( \dfrac{\text{LC}_{50}}{100} )</td>
<td>27.63 ± 2.40 (∆29.80)</td>
<td>22.61 ± 1.86 (∆44.17)</td>
<td>16.49 ± 1.28 (-61.04)</td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean of six individual observations ± S.E; Minus values denoting % decrease from control level are given in parentheses

Table 2: Acetyl cholinesterase activity (μ moles of acetylcholine hydrolysed/mg protein/h) in the brain of control \( O. mossambicus \) and that exposed to sublethal concentrations of Thiodan

<table>
<thead>
<tr>
<th>Days</th>
<th>Concentration</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.34 ± 0.66</td>
<td>11.20 ± 0.54</td>
<td>11.14 ± 0.47</td>
<td></td>
</tr>
<tr>
<td>( \dfrac{\text{LC}_{50}}{10} )</td>
<td>10.45 ± 0.65 (∆7.84)</td>
<td>10.17 ± 0.58 (∆9.19)</td>
<td>9.49 ± 0.30 (-16.82)</td>
<td></td>
</tr>
<tr>
<td>( \dfrac{\text{LC}_{10}}{10} )</td>
<td>9.68 ± 0.29 (∆14.63)</td>
<td>7.53 ± 0.63 (-32.76)</td>
<td>6.33 ± 0.47 (-44.52)</td>
<td></td>
</tr>
<tr>
<td>( \dfrac{\text{LC}_{50}}{100} )</td>
<td>7.45 ± 0.62 (-34.30)</td>
<td>5.66 ± 6.33 (-49.46)</td>
<td>3.48 ± 0.45 (-69.50)</td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean of six individual observations ± S.E; Minus values denoting % decrease from control level are given in parentheses
in AChE level might also disrupt the endocrine functioning of the fish. So it is clear that these responses can be used as a tool in biomonitoring programme to screen ecotoxicity risk of endosulfan to the test species.

REFERENCES


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