INDEPENDENT AND JOINT TOXICITY OF FEW INSECTICIDES ON FRESHWATER CATFISH, *HETEROPNEUSTES FOSSILIS*

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** ABSTRACT:** Indiscriminate use of insecticides in the agricultural fields to control insect pests greatly enhances their potential in aquatic ecosystems which affects the fishes also. Fishes get exposed to several toxicants at a time in natural water bodies. The present piece of work is an attempt to understand the independent and joint acute lethal toxicity of two compounds, viz. Quinalphos (an organophosphate Insecticide) and Cypermethrin (a synthetic pyrethroid) on the freshwater fish, *Heteropneustes fossilis* under laboratory conditions. The 96 hr LC₅₀ (median lethal concentration) values of Quinalphos, Cypermethrin and their mixture (in 1:1 ratio) were 6.333 mg/L, 0.280 mg/L and 0.410 mg/L respectively. It was found that Cypermethrin was highly toxic than Quinalphos + Cypermethrin combination and Quinalphos to catfishes under static renewal 96 hr LC₅₀, bioassay. The joint or combined toxicity tests revealed the “less than additive” interaction which indicates moderate antagonism.

**Key words:** Lethal toxicity, Quinalphos, Cypermethrin, *Heteropneustes fossilis*

### INTRODUCTION:

An increased usage of several insecticides is an attempt to control the insect pests that destroy the crops in agricultural fields. It has resulted in the development of resistance by the target organisms to the pesticides. The insecticides reach to the water bodies through translocation. Thus, a polluted freshwater ecosystem harbours different pesticides at different concentrations, forming a pool of pesticides, where the toxicity of each pesticide is considerably modified by the presence of other pesticides. The interaction occurs amongst pesticides and abiotic factors influencing the ecosystem. Therefore, the studies on the interaction among toxicants are required to understand the actions of combined (or joint) toxicity such as ‘antagonism’ in which the interaction reduces the toxicity of insecticide and ‘synergism’ in which it increases the toxicity of insecticide (Wilkinson, 1976). The combined effects of pesticides on aquatic animals are studied by Durairaj and Selvaraj (1995), Sambasiva Rao (1999), Deneer (2000), Jha and Verma (2002), Nair *et al.* (2007), and Muthulakshmi *et al.* (2008).

The present piece of work done to know the independent and joint acute toxicity of Quinalphos and Cypermethrin on the freshwater catfish, *Heteropneustes fossilis* is found to be a suitable test animal for toxicity monitoring. Acute toxicity is a relatively short-term lethal or other effect of a substance usually occurring within 4 days (APHA-AWWA-WPCF, 1985).

### MATERIALS AND METHODS:

**Test animals:** The living freshwater air-breathing catfish, *Heteropneustes fossilis* were collected from local fish markets of Nagpur city. The fishes of similar size and weight ranging from 18.8 to 20.2 cm in total length and 40.0 to 60.0 gm in weight were chosen for toxicity tests. They were acclimated to the laboratory conditions by using large glass aquaria containing water for a period of 10 days prior to the start of the experiments. During acclimatization, fishes were fed on artificial feed (Tokyo pellets) once a day. The toxicity of test material may be influenced by physical and chemical properties of the dilution water. Hence, these parameters were analysed by following standard methods (APHA-AWWA-WPCF, 1976).

**Test chemicals:** Two dissimilar insecticides, viz. Quinalphos 25% EC (an organophosphate) and Cypermethrin 25% EC (a synthetic pyrethroid), and their mixture (in 1:1 ratio) were used for bioassay tests as per method described below.

**Stock solution:** Stock solutions of Quinalphos, Cypermethrin and their mixture (in 1:1 ratio) were prepared by dissolving 1.0 ml of each toxicant in dilution water separately and raised the volume to 1 litre as these toxicants are soluble in water. 1 ml of prepared stock solution contains 1 mg of toxicant (hence, concentration of the original toxicant was assumed to be 1.0 gm/cc). Appropriate quantity of stock solution of particular toxicant was added to dilution water to prepare required different concentrations. Stock solutions were freshly prepared every day.

**Determination of lethal toxicity:** Bioassay technique is used for detection of lethal toxicity as per standard methods by APHA-AWWA-WPCF (1976). To determine acute lethal toxicity (96 hr LC₅₀), range finding tests were conducted by static with renewal bioassay procedure by using 10 fishes per test concentration and their definitive tests were carried out within the narrow range. Tests were carried out by following the completely randomized design in glass aquaria with 40 litre capacity in triplicate. Test solutions were renewed once in every 24 hours. A control was also maintained for each set. Mortality during the 96 hr exposure was recorded for each treatment. 96 hr LC₅₀ values, their 95% confidence limits and slope of probit regression line were evaluated by Probit Analysis (Finney, 1971) using SPSS statistical package. By same way as in independent toxicity, screening tests were carried out for joint toxicity of two different toxicants (i.e. mixture) and then definitive tests were conducted within narrow range. The median lethal concentration of the mixture is determined by following probit analysis. For joint toxicity, the method suggested by Sprague (1973) is adopted. Joint or combined effects of the components of mixture appear in the form of more-than-additive, less-than-additive or additive interaction indicating synergism or antagonism or an independent effect, respectively.  

The strength of test material is calculated as follows:

\[
\text{Toxic units} = \frac{\text{Actual concentration in solution}}{\text{Lethal threshold concentration (LC₅₀)}}
\]
For a mixture, the number of toxic units is calculated for each of the component toxicants. Since, the strength of all are expressed in the same units, they are added together (EIFAC, 1980). If the total of toxic units is 1.0 or larger, the mixture is predicted to be lethal.

RESULTS AND DISCUSSIONS:

Physico-chemical parameters of the dilution water were assessed. Temperature ranged from 26°C to 28°C, pH from 7.4 to 8.0, dissolved oxygen from 7.0 to 7.4 mg/L and hardness from 160 to 176 mg/L as CaCO₃.

Final results of the probit analysis are summarized in Table 1. The 96 hr LC₅₀ values to catfishes are 6.333 mg/L for Quinalphos, 0.280 mg/L for Cypermethrin, and 0.410 mg/L for Quinalphos + Cypermethrin mixture (in 1:1 ratio). Cypermethrin is found to be most toxic followed by mixture and Quinalphos. LC₅₀ values of insecticides and their 95% confidence limits are represented graphically in Figs. 1, 2 & 3.

The 96 hr LC₅₀ value of both insecticides and their mixture lie within its 95% confidence limit with a probability level of 0.95. The regression coefficient (slope) of probit regression line shows a positive relationship between the concentration and mortality which is tested by t-test for linearity of regression and is found significant at P < 0.002 for Quinalphos, P < 0.003 for Cypermethrin and P < 0.004 for the mixture. The fitted model is assessed by statistics of heterogeneity which follows a chi-square distribution. Chi-square is not significant that indicates the line is a good fit (i.e., an adequate representation of the data). The linear relationship between probit mortality and dose can be expressed for Quinalphos, Cypermethrin and their combination as 

\[ Y = 12.7699 + 15.9308X \]

\[ Y = 3.4050 + 6.1648X \]

\[ Y = 2.2561 + 5.8278X \]

For a mixture, the combined action of two different insecticides (Quinalphos and Cypermethrin) is presented in Table 2. The total of toxic units is 0.7646 for Quinalphos + Cypermethrin combination (in 1:1 ratio) in which Quinalphos contributes 0.0324 toxic unit and Cypermethrin contributes 0.7321 toxic unit. Hence, the total of toxic units is found to be less than 1.0 toxic unit showing "less than additive" interaction one with other toxicant which indicates moderate antagonism.

Many workers carried out the experiments to assess the acute toxicity of various pesticides to different fishes. The results of the present study on acute toxicity are compared with the available literature by Gouda et al. (1981), Singh and Singh (1983), Plaha (1988), Lonkar (1996), Dixit et al. (2004), Mercy et al. (2001), Chandrahekaran et al. (2005), and Charjan et al. (2008). Regarding joint toxicity of Quinalphos and Cypermethrin, results are also compared with the work done by Sambasiva Rao (1999), Nair et al. (2007), and Muthulakshmi et al. (2008).

All these studies show that the toxicity of pesticides to fish are dose dependent. Mortality depends on the concentration and duration of exposure. It also reveals that different fishes have different tolerance capacity against toxic effects of same pesticide and toxicity of different pesticides varies among the same species depending on size, physico-chemical parameters and density in terms of number of specimens used.

The general conclusion, which can be drawn from the present investigation is the insecticide, Cypermethrin is more toxic to the catfish, Heteropneustes fossilis than the mixture and Quinalphos. The joint action of both insecticides exhibited the "less-than-additive" interaction that indicates moderate antagonism.

### Table 1: Acute toxicity of selected insecticides to Heteropneustes fossilis

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Aquatic species</th>
<th>Insecticides</th>
<th>96 hr. LC₅₀ (mg/L)</th>
<th>95% confidence limits of LC₅₀</th>
<th>Slope of probit regression line</th>
<th>‘t’ for regression</th>
<th>Probability (P)</th>
<th>Chi² for response</th>
<th>Probability (P)</th>
<th>Significance</th>
<th>Regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Heteropneustes fossilis</td>
<td>a) Quinalphos</td>
<td>6.333</td>
<td>5.9918 - 6.6820</td>
<td>15.9308</td>
<td>5.3749</td>
<td>0.0017</td>
<td>1.7847</td>
<td>0.9384</td>
<td>NS</td>
<td>Y = -12.7699 + 15.9308X</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Cypermethrin</td>
<td>0.280</td>
<td>0.2425 - 0.3204</td>
<td>6.1648</td>
<td>4.9169</td>
<td>0.0027</td>
<td>2.3871</td>
<td>0.8809</td>
<td>NS</td>
<td>Y = 3.4050 + 6.1648X</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) Mixture (Q+C)</td>
<td>0.410</td>
<td>0.3464 - 0.4712</td>
<td>5.8278</td>
<td>4.5000</td>
<td>0.0041</td>
<td>3.5696</td>
<td>0.7347</td>
<td>NS</td>
<td>Y = 2.2561 + 5.8278X</td>
</tr>
</tbody>
</table>

### Table 2: Joint toxicity of selected insecticides to Heteropneustes fossilis for 96 hr exposure

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Aquatic animals</th>
<th>Insecticide</th>
<th>Ratio of combination</th>
<th>96 hr. LC₅₀ (mg/L)</th>
<th>95% confidence limits of LC₅₀</th>
<th>Toxic Units</th>
<th>Joint toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Heteropneustes fossilis</td>
<td>a) Quinalphos</td>
<td>-</td>
<td>6.333</td>
<td>5.9918 - 6.6820</td>
<td>0.0324</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Cypermethrin</td>
<td>-</td>
<td>0.280</td>
<td>0.2425 - 0.3204</td>
<td>0.7261</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) Quinalphos + Cypermethrin</td>
<td>1:1</td>
<td>0.410</td>
<td>0.3464 - 0.4712</td>
<td>0.7645</td>
<td>Less than additive</td>
</tr>
</tbody>
</table>

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