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Full Length Research Paper

Acute effects of cybermethrine as potential dangerous additives on common carp (Cyprinus carpio)

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Cypermethrine is one of the most dangerous poisons that are commonly used in agricultural purpose. The aims of the present study are to investigate acute effects of cybermethrine as potential additives of water ecosystems to assess mortality effects of these chemical products on main cultured warm-water fish of Iran. Common Carp (*Cyprinus carpio*) was exposed to the cypermethrine (0, 0.5, 2, 4, 8 and 16 ppm). LC_{50} was determined with probit analysis. The 96 h toxicity tests showed 100% mortality in 8 and 16 ppm, while in all treatments one mortality was observed. As it was found that LC_{50} of cypermethrine in common carp (1.91 ppm) was lower than that of other species, it means there is resistance of common carp in comparison with other species. Eventually, the findings of this study indicate that LC_{50} cypermethrine is more toxic to cultured fish and it should be moderately used for agricultural purpose.

Key words: Fish, LC_{50}, pollution, poison, toxicity test.

INTRODUCTION

Acute toxicity data can help identify the mode of toxic action of a substance and may provide information on doses associated with target-organ toxicity and lethality that can be used in setting dose levels for repeated-dose studies. This information may also be extrapolated for use in the diagnosis and treatment of toxic reactions in humans. The results from acute toxicity tests can provide information for comparison of toxicity and dose-response among members of chemical classes and help in the selection of candidate materials for further work (Hedayati et al., 2010a).

Lethal concentration (LC_{50}) is the ambient aqueous chemical activity that causes 50% mortality in an exposed population. These calculations are based on two important assumptions. The first assumption is that the exposure time associated with the specified LC_{50} is sufficient to allow almost complete chemical equilibration between the fish and the water. The second assumption is that the specified LC_{50}, the minimum LC_{50} kills the fish during the associated exposure interval. Fortunately, most reliable LC_{50} satisfy these two assumptions (Boudou and Ribeyre, 1997).

The 96-h LC_{50} tests are conducted to measure the susceptibility and survival potential of organisms to particular toxic substances such as oil pollution. Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in organisms (Eisler and Gardener, 1993).

Petroleum products are one of the most relevant of aquatic ecosystems. Today, little research has been done on the effects of petroleum products on fishes (Di Giulio and Hinton, 2008), also considering the growing cases of environmental accidents involving spills of petroleum distillate products into continental waters in the last years into the urban waters. The aims of the present study are to investigate acute effects of cybermethrine as potential dangerous additives to assess mortality effects of these chemical products on valuable cultured fish of Iran, Common Carp (*Cyprinus carpio*).

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Table 1. Cumulative mortality of common carp during acute exposure to cybermethrine (n=21, for each concentration).

Concentration (ppm)	No. of mortality			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
0.5	0	0	1	8
2	0	5	9	11
4	8	11	17	18
8	11	12	18	21
16	21	21	21	21

MATERIALS AND METHODS

Acute toxicity tests were conducted on common carp (~ 15 g and 11 cm) purchased from private warm water fish farm in Gonbad, Iran. Only healthy fish, as indicated by their activity and external appearance, were maintained alive on board in a fiberglass tank. Samples transferred to a 400-L aerated tank equipped with aeration with 200 L of test medium.

All samples were acclimated for one week in a 15 aerated fiberglass tank at 25°C under a constant 12:12 L:D photoperiod. Acclimatized fish were fed daily with a formulated feed (Chineh, Iran). Dead fish were immediately removed with special plastic forceps to avoid possible deterioration of the water quality (Gooley et al., 2000).

Laboratory cypermethrine tested concentrations were 0, 0.5, 2, 4, 8 and 16 ppm. Groups of 21 fish were exposed for 96 h in fiberglass tank. Test medium was not renewed during the assay and no food was provided to the animals during the test. Values of mortalities were measured at time 0, 24, 48, 72 and 96 h (Hedayati et al., 2010b).

Acute toxicity tests were carried out in order to calculate the 96h-LC_{50} for cypermethrine, based on Hotos and Vlahos (1998). Mortality was recorded after 24, 48, 72 and 96 h. The LC_{50} values and its confidence limits (95%) were calculated by Boudou and Ribeyre (1997). Percentages of fish mortality were calculated for each cypermethrine concentration at 24, 48, 72 and 96 h of exposure.

LC_{50} values were calculated from the data obtained in acute toxicity bioassays, by Finney's (1971) method of "probit analysis" and with SPSS computer statistical software. In Finney's method, the LC_{50} value is derived by fitting a regression equation arithmetically and also by graphical interpolation by taking logarithms of the test chemical concentration on the X axis and the probit value of percentage mortality on the Y axis (Finney, 1971).

The 95% confidence limits of the LC_{50} values obtained by Finney's method were calculated with the formula of Mohapatra and Rengarajan (1995). Probit transformation adjusts mortality data to an assumed normal population distribution that results in a straight line. Probit transformation is derived from the normal equivalent deviate (NED) approach developed by Tort et al. (1988), who proposed measuring the probability of responses (that is, proportion dying) on a transformed scale based on the percentage of population or the standard deviations from the mean of the normal curve (Di Giulio and Hinton, 2008).

The $LC_{1,10,30,50,70,90,99}$ values were derived using simple substitution probit of 1, 10, 30, 50, 70, 90 and 99 respectively for probit of mortality in the regression equations of probit of mortality vs. cypermethrine. The 95% confidence limits for LC_{50} were estimated by using the formula:

LC_{50} (95% CL) = LC_{50} ± 1.96 [SE (LC_{50})].

The SE (Standard Error) of LC_{50} is calculated from the formula:

$$SE(LC50) = 1/b\sqrt{pnw}$$

Where: b = the slope of the cybermethrine/probit response (regression) line; p = the number of cybermethrine used; n = the number of animals in each group; w = the average weight of the observations (Hotos and Vlahos, 1998).

At the end of the acute test, the LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) were determined for each endpoint measured. In addition, the maximum acceptable toxicant concentration (MATC) was estimated for the endpoint with the lowest NOEC and LOEC (Hedayati et al., 2011).

RESULTS AND DISCUSSION

The mortality of studied fishes for cypermethrine doses 0, 2, 5, 10, 20 and 40 ppm were examined during the exposure times at 24, 48, 72 and 96 h (Table 1). Fishes exposed during the period of 24-96 h had significantly increased number of dead individual with increasing concentration ($P<0.05$). There were significant

Table 2. Lethal concentrations (LC_{1-99}) of cypermethrine (mean ± standard error) depending on time (24-96 h) for common carp.

Point	Concentration (ppm) (95% of confidence limits)			
	24 h	48 h	72 h	96 h
LC_1	0.001 ± 0.3	0.001 ± 0.5	0.001 ± 0.3	0.001 ± 0.6
LC_{10}	2.90 ± 0.33	0.99 ± 0.54	0.02 ± 0.39	0.004 ± 0.6
LC_{30}	5.31 ± 0.33	3.89 ± 0.54	2.03 ± 0.39	0.98 ± 0.63
LC_{50}	**6.98 ± 0.33**	**5.89 ± 0.54**	**3.42 ± 0.39**	**1.91 ± 0.63**
LC_{70}	8.64 ± 0.33	7.89 ± 0.54	4.81 ± 0.39	2.84 ± 0.63
LC_{90}	11.0 ± 0.33	10.7 ± 0.54	6.82 ± 0.39	4.10 ± 0.63
LC_{99}	14.3 ± 0.33	14.78 ± 0.5	9.59 ± 0.39	6.04 ± 0.63

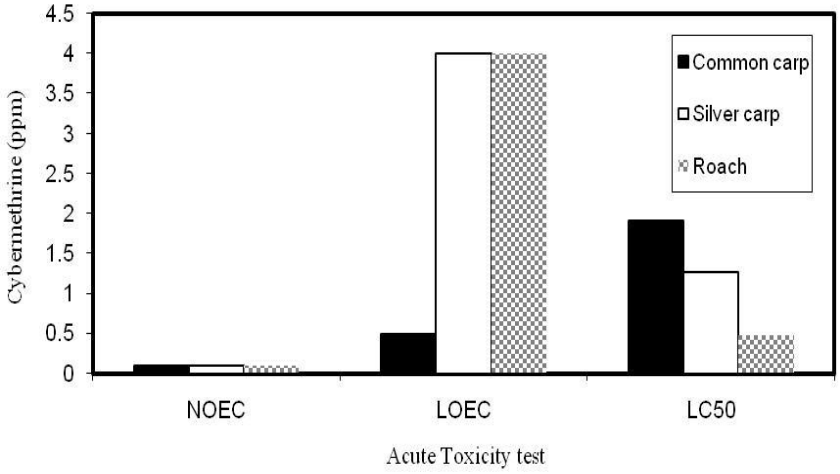

Figure 1. Acute toxicity testing statistical endpoints of common carp exposed to 96 h acute toxicity test of cybermethrine.

differences in the number of dead fish between the duration of 24-96 h in each sample ($P<0.05$). There were 100% mortality at 40 ppm concentration within the 96 h after dosing for all fishes, and no mortality at 2 and 5 ppm within the exposure times for all species.

Median lethal concentrations of 1, 10, 30, 50, 70, 90 and 99% test are shown in Table 2. These were time-mortality lines. It was found that LC_{50} of cypermethrine in common carp was higher than that of other species (Figure 1).

Toxicity Testing Statistical Endpoints are shown in Figure 1 (Shaluei et al., 2012). LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) were same for all studied fishes, however LC_{50} (the median lethal concentration) had significant different between species (Shaluei et al., 2012). The Maximum Acceptable Toxicant Concentration (MATC) for common carp was 0.191 ppm of cypermethrine respectively.

In determining the toxicity of a new chemical to fish, an acute toxicity test is first conducted to estimate (LC_{50}) of the chemical in water to which organisms are exposed (Di Giulio and Hinton, 2008). The relationship between the degree of response of test organisms and the quantity of exposure to the chemical almost always assumes a concentration–response form. As in the results of this study, the *y*-axis represents percentage mortality and the *x*-axis represents concentration of cybermethrine. Both variables increased with distance from origin. The cumulative responses to cybermethrine concentrations yield the sigmoid (S-shaped) curve (Di Giulio and Hinton, 2008).

Variability in acute toxicity even in a single species and single toxicant depends on the size, age, and condition of the test species along with the experimental factors. The differences in acute toxicity may be due to changes in water quality and test species (Rathore and Khangarot, 2002).

In the present study, LC_{50} values indicated that cybermethrine is more toxic to cultured fish. LC_{50} obtained in the present study, when compared with the corresponding values that have been published in the

literature for other species of fish, showed different LC_{50} of cybermethrine in different species and even different time, but lower value of LC_{50} for the studied fish was important, thus confirming sensitivity of aquaculture species to low cybermethrine doses. Although the LC_{50} under a defined set of environmental conditions can provide useful information, the numeric value cannot be used in the field; so in continuation of this study, we suggest the use of some biomarkers for better understanding of cybermethrine toxicity.

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