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EFFECT OF SOME ENVIRONMENTAL FACTORS ON THE ACUTE TOXICITY OF DELTAMETHRIN TO COMMON CARP: A LABORATORY STUDY UNDER AEROBIC CONDITION

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ABSTRACT

The toxicity of xenobiotics in aquatic ecosystems is influenced by many factors such as ambient temperature, water hardness, pond soil type, etc. In the present study, it was observed that air temperature, water hardness and soil sediment have profound influence on the toxicity of deltamethrin to common carp fry (av. length 3.5 ± 0.5 cm, av. weight 0.58 ± 0.25 g); 96h LC_{50} values for common carp at 38.07 ± 2.20° C maximum and 27.86 ± 1.22° C minimum air temperature in soft and very hard water were 0.102 and 0.495 µg l^{-1}, respectively. This value had increased significantly to 2.37 and 3.02 µg l^{-1} at 30.55 ± 1.21° C maximum and 26.04 ± 0.61° C minimum air temperature, respectively. When sediment was included, 96h LC_{50} at 38.07° C maximum temperature in very hard water was 1.808 µg l^{-1} and this had increased to 8.073 µg l^{-1} when tested at 30.55° C maximum temperature. Due to the 7.5° C increase in maximum and 1.7° C in minimum temperature, toxicity increased significantly. Lower toxicity in very hard water in comparison to soft water may be due to the lower solubility of deltamethrin and high level of calcium. Adsorption reaction of deltamethrin with clay, humus, FeOOH, MnOOH and particulate organic carbon, and complexation reaction with dissolved organic carbon were responsible for the lowered toxicity in the experiment with sediment. Exposure time had no significant effect on acute toxicity of deltamethrin.

Keywords: Deltamethrin, common carp, temperature, water hardness, sediment

INTRODUCTION

Deltamethrin, a synthetic pyrethroid, has been in use for more than two decades now. It was first marketed in 1977 and used widely for the control of various pests of cotton, coffee, maize, cereals and vetetables; also used in public health programme and protection of stored crops (IARC, 1991). In most of the commercially formulated pesticides, deltamethrin has been used as an active ingredient. At present, deltamethrin and allied pyrethroids

(*e.g.*, cypermethrin, permethrin, fenvelerate) have been widely in use as spray for agricultural and horticultural crops on account of their low mammalian toxicity, wide spectrum of activity on the pest and low application rates. But the extensive use of these pesticides in the long run will lead to the increase in toxic effect on aquatic organisms and other higher animals. Some of the recent studies on environmental effects have revealed certain drawbacks in their applications. Aquatic organisms, particularly fish, are highly sensitive to pyrethroids (Stephenson, 1982; Smith and Stratton, 1986; Coats *et al.*, 1989; Haya, 1989; Reddy and Bashamohideen, 1989; Malla-Reddy *et al.*, 1995). Few reports also pointed out the highly toxic effect of deltamethrin to aquatic organisms, mostly fishes (WHO, 1990; Salyi and Casaba, 1994; Nemesok *et al.*, 1999).

As aquatic ecosystems act as a sink for all types of pollutants, there is a need to assess the relative environmental hazards of these potential contaminants in aquatic ecosystems. Water is the medium where fish live. The water body stands on soil. Majority of the studies related to the establishment of the toxicological effect of a chemical to fish did not include the soil part, though it is a must in the natural condition. Different physicochemical parameters of water and soil can affect the toxicity of chemicals in aquatic ecosystems (Datta and Das, 2002; Datta *et al.*, 2001, 2002a, 2002b, 2002c; Datta, 2003). In the present study, deltamethrin, which has a sufficiently high mammalian safety ratio (6000), was tested for its toxic effect on *Cyprinus carpio* var. *communis* under the influence of air temperature, water hardness, sediment and exposure time.

MATERIAL AND METHODS

The test chemical used for the study was Decis®, containing 2.8% deltamethrin as emulsifiable concentrate. Advanced fry of common carp, *Cyprinus carpio* var. *communis* (av. length: 3.5 ± 0.5 cm and av. weight: 0.65 ± 0.25 g), was selected for investigation because of its importance in freshwater aquaculture all over the world and its availability throughout the year. Cylindrical plastic buckets and glass jars of 20 l capacity were used as test containers for water and water with sediment experiments, respectively. Simple aquarium aerators with two outlets purchased from the local market were used for aeration. Experiment was conducted at two levels of water hardness, *i.e.*, soft water (0.90 m*M* $Ca^{2+}l^{-1}$). Soft water was prepared by mixing distilled water (0.10 m*M* $Ca^{2+}l^{-1}$ total hardness) and drinking water (1.70 m*M* Ca^{2+} l^{-1} total hardness) in the ratio 5:5. For very hard water, tap water (7.2 m*M* $Ca^{2+}l^{-1}$) was used directly.

Two kinds of colloidal materials, inorganic (clay) and organic (humus) were used for the experiment with sediment. For clay particles, soil was collected from different pollution-free areas of West Bengal and finally, soil from 24 Parganas (North), which contains 24.88% clay, 21.00% silt and 3.01% organic matter was used. For humus, well decomposed, dried, powered, farmyard manure was used. Healthy, disease-free common carp fry were brought to the laboratory from Fish Cultivate Centre, Naihati, and the work was carried out following standard procedures described (APHA, 1998).

Continuous aeration was given to maintain the aerobic condition with

dissolved oxygen level between 7 and 8 mg l^{-1}. Four treatments of water, *i.e.*, two treatments in soft water (T1 and T4) at two different temperatures and two treatments in very hard water (T2 and T5) at two different temperatures were conducted. Five to eight concentrations, three replications per concentration and 10 l of test water in each replication were taken. Fry were exposed under static conditions for 96 hours during the test. The fish were not fed. Parameters such as dissolved oxygen, free CO_2, pH, total alkalinity, total hardness, PO_4^{3-}, NO_3^-, NO_2^-, NH_4^+ and salinity were measured daily at the same time. Maximum and minimum ambient temperatures in the laboratory during two phases (March-April and July-August 2002) of the experiment were recorded daily. Mortality data were taken at every 12-hour interval, and the fish were counted as dead when there was no movement and found at the bottom of the containers. Along with mortality, the behavioural changes of fry were also recorded following standard methods (APHA, 1998).

One treatment of sediment with very hard water was also maintained for each temperature (T3 and T6). Hardness of water in treatment with sediment was kept the same as that of the corresponding water treatment for comparison of the effect. To get realistic levels of particulate and dissolved organic carbon in aqueous phase, 100 g powdered sediment (97 g soil and 3 g humus) was introduced into 10 l of test water, stirred with a glass rod and desired concentration of toxicant mixed. The mixture was kept for eight hours for the settling of the sediment at the bottom of the glass jar and to increase the transparency of this solution (20-40 cm) before the fish were released.

The physicochemical parameters like total hardness, pH, dissolved oxygen, PO_4^{3-}, NO_3^-, NO_2^- and NH_4^+ were measured just before and during the experiment using Aquamerck Compact Laboratory (E. Merck, Germany). For free CO_2, total alkalinity and chlorinity, titrimetric methods were used. Textural analysis of soil was carried out by soil hydrometer. Per cent organic carbon and per cent organic matter of the soil sample were estimated following the Wakley and Black method (Chopra and Kanwar, 1991). Particulate and dissolved organic carbon in the aqueous phase was also estimated by modifying the Wakley and Black method. For the estimation of dissolved organic carbon (DOC), water samples from the experiments with sediment were collected and filtered to remove the particulate matter. DOC was estimated in 200 ml of filtered water with 10 ml 1*N* $K_2Cr_2O_7$, 20 ml conc. H_2SO_4, 20 ml H_3PO_4, 1 ml biphenyl amine indicator and 0.5*N* $FeSO_4$ solution. For the estimation of particulate organic carbon (POC), 50-ml water samples were collected and used for the test without filtration: 150 ml of distilled water was added to it and 1*N* $FeSO_4$ solution was used for titration. DOC was calculated from the equation 0.5 X (volume of 0.5*N* $FeSO_4$ consumed by the blank – volume of 0.5 *N* $FeSO_4$ consumed by the filtered water) X 3 X 5, which was estimated to be 7.5 ± 1.5 mg l^{-1}. Total organic carbon (TOC) of the water sample was calculated from the equation {(volume of 1*N* $FeSO_4$ consumed by the blank – volume of 1*N* $FeSO_4$ consumed by the filtered water) X 3 X 20} mg C l^{-1}. The

difference between TOC and DOC was referred to as particulate organic carbon (Kolka *et al.*, 1999), which was maintained at 22 ± 2.0 mg l^{-1}. Per cent mortality of fry was transformed into probity values and plotted along the Y-axis. Log_{10} values of different concentrations were plotted along the X-axis to obtain linear regression equations and regression coefficients. LC_{50} values at 24, 48, 72 and 96 hours and 95% confidence limits for the LC_{50} values were calculated from regression equations (Finney, 1971), $LC_{0.1}$, 95% confidence limits for 96h LC_{50} values and safe application rate (SAR) at different conditions (Basak and Konar, 1977) were also calculated. Analysis of variance (ANOVA) and Student's 't' test were carried out to find the significance between different treatments. MS Excel 2000 in Windows 98 computer operating system was used to obtain regression equation and regression coefficients, and for the analysis of variance and Student's 't' test. For statistical analysis, 24h, 48h, 72h and 96h LC_{50} data were taken as four replications in each condition, and average of these four replications was taken as average LC_{50} value of that particular condition. For testing the effect of exposure time on toxicity, LC_{50} values of all the conditions at every 24-hour interval were taken as replications and the average values were taken as average LC_{50} value of the particular exposure time.

RESULTS AND DISCUSSION

PO_4^{3-}, NO_3^-, NO_2^- and NH_4^+ values (Table 1) were not subjected to statistical analysis as the exact value could not be determined by the kit. Other parameters did not show any significant difference between the control and treated sets when tested at 5% level of significance using Fisher's 't' test.

Table 1: Physicochemical parameters (mean ± SD, n = 24) of different kinds of water used for the experiment

Parameter	Control		Treatment	
Total hardness (m*M* l^{-1})	0.9 ± 0.05	7.2 ± 0.10	0.9 ± 0.05	7.2 ± 0.10
Total alkalinity	94 ± 2.0	376 ± 4.0	94 ± 2.0	376 ± 4.0
Cl^- (%)-	0.03 ± 0.01	0.55 ± 0.01	0.03 ± 0.01	0.55 ± 0.01
pH	7.7 ± 0.1	7.9 ± 0.1	7.7 ± 0.1	7.9 ± 0.1
O_2 (mg l^{-1})	8.0 ± 0.3	7.4 ± 0.4	7.8 ± 0.3	7.2 ± 0.3
CO_2 (mg l^{-1})	5.5 ± 1.0	10.0 ± 2.0	6.5 ± 1.5	11.3 ± 2.0
NO_3^- (mg l^{-1})	0	25 - 50	0	25 - 50
PO_4^{3-} (mg l^{-1})	0	0 - 0.25	0	0 - 0.25
NO_2^- (mg l^{-1})	0	0 - 0.025	0	0 - 0.025
NH_4^+ (mg l^{-1})	0	0 - 0.2	0	0 - 0.2

Behavioural changes were significantly prominent at higher concentrations of the test material. During the initial stages of the experiment, fish exhibited erratic swimming behaviour and dashed to the sides of the containers. As the test period prolonged, activity of fishes was reduced greatly. Fishes surfaced frequently to gulp air, followed by sideward movements. During the later stages of experiment, fry swarm to the upper layer and remained lethargic, respiring slowly prior of death.

Acute toxicity data of deltamethrin under different conditions are presented in tables 2 and 3. Regression equations, regression coefficients (R^2), 96h LC_{50} values and safe application rate were calculated under all conditions (Fig. 1) and the 95% confidence limits for 96h LC_{50} values (as Y-axis bar) and 96h LC_{50} are presented in Fig. 2. The results of statistical analyses are given in Table 3 after comparing the mean LC_{50} values in different treatments with the least significant difference by t-test at 5%. It was observed that lowering air temperature, increasing water hardness and inclusion of soil sediment decrease the acute toxicity of deltamethrin to common carp fry significantly. But, exposure time up to 96 hours has no significant effect.

Table 2: Acute toxicity of deltamethrin to common carp fry

Parameter	Soft water treatment		Very hard water treatment		Very hard water with sediment treatment	
	T1 38.07° C	T4 30.55° C	T2 38.07° C	T5 30.55° C	T3 38.07° C	T6 30.55° C
Regression equation	Y = 4.754X +0.199	Y = 4.362X -0.979	Y = 3.310X -0.613	Y = 4.183X -1.193	Y = 6.819X -10.390	Y = 6.633X -1.017
Regression coefficients	0.96	0.78	0.87	0.86	0.67	0.80
96h LC_{50} ($\mu g\ l^{-1}$)	0.102	2.373	0.495	3.023	1.808	8.073
SAR ($\mu g\ l^{-1}$)	0.024	0.506	0.064	0.603	0.673	2.932

Table 3: Results of statistical analyses

Test	Fcal	Fcrit	*P*-value	Average LC_{50} ($\mu g\ l^{-1}$)	Lsdt at 5%	Test result
Effect of temp. and sediment	97.46	2.77	2.17E-12	T1, 0.107; T2, 0.676 T3, 2.474; T4, 2.954 T5, 3.976; T6, 9.412	1.0043	Temp.: T4>T1 T5>T2 T6>T3 Sediment: T6>T5 T3>T1
Effect of water hardness	64.11	3.49	1.17E-07	T1, 0.107; T2, 0.676 T4, 2.954; T5, 3.976	0.534	T2>T1 T5>T4
Effect of exposure time	0.143	3.09	0.932	24 h, 3.85; 48 h, 3.48 72 h, 3.08; 96 h, 2.64	-	Non-significant

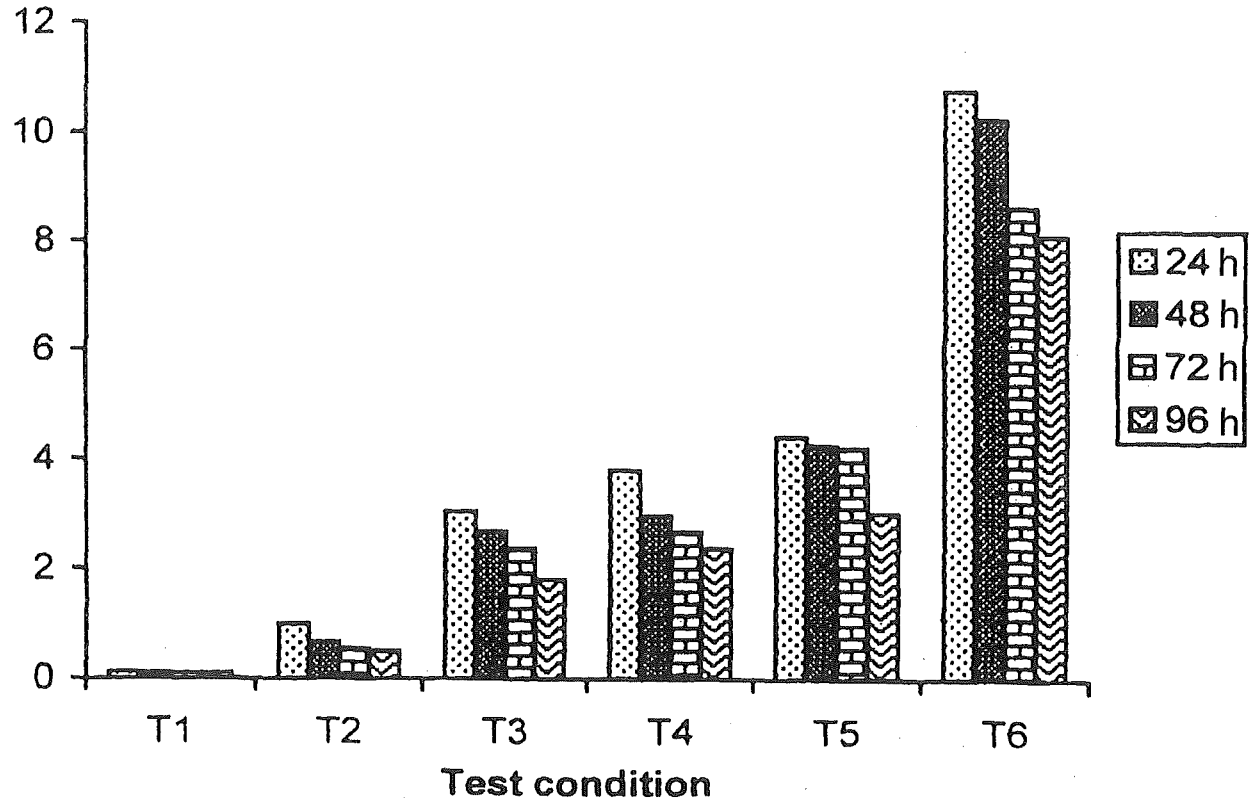

Fig. 1. LC_{50} values of deltamethrin under different test conditions at different times

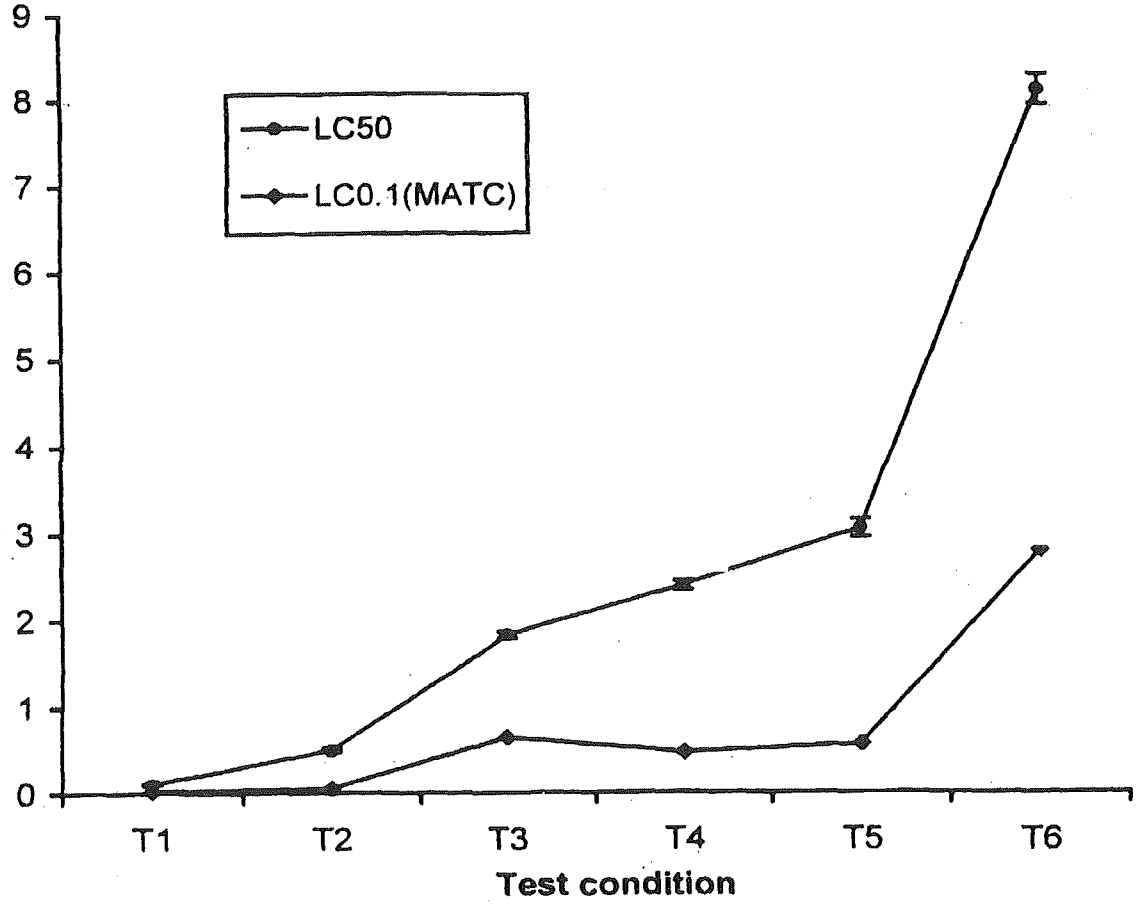

Fig. 2. LC_{50} (with 95% confidence limits) and $LC_{0.1}$ values under different test conditions

It was observed that the regression coefficients in different conditions were in the range of 0.67 to 0.97, which signifies a good correlation between observed and expected data. Air temperature had significant influence in modifying the acute toxicity of deltamethrin to common carp fry. It was found that at lower temperature, acute toxicity was significantly lower (higher LC_{50}) in all the conditions (Table 2). Due to the 7.5°C reduction in maximum temperature, the acute toxicity of deltamethrin to common carp fry in August 2002 was significantly lower. In August 2002, average maximum and minimum temperatures were 30.55 ± 1.21 and 26.04 ± 0.61° C; and 96h LC_{50} and SAR were 2.373 and 0.506, 3.023 and 0.603, and 8.073 and 2.932 µg/l, respectively (Fig. 1). The 96h LC_{50} values (Fig. 2) were also in accordance with the above trend. There was no probity value below 0.1% mortality rate. So, $LC_{0.1}$ value can be treated as the lowest concentration where mortality just starts. It was observed that $LC_{0.1}$ values were lower than the SAR values. In T1, T2, T3, T4, T5 and T6, 96h $LC_{0.1}$ values were 0.022, 0.057, 0.636, 0.459, 0.551 and 2.762 µg l^{-1}, respectively, which were slightly lower than the 96h SAR values (Table 2). Therefore, in the present experiment, $LC_{0.1}$ values were taken as the maximum allowable toxic concentration for 96 hours in different treatment conditions and are plotted with LC_{50} values in Fig. 2. Increased temperature facilitates increased uptake of water, ions and other dissolved substances by fish and thereby increased toxicity of many pollutants (WHO, 1992). This might be the reason for the significantly higher toxicity of deltamethrin at higher temperature, *i.e.*, average maximum 38.07 ± 2.20°C and minimum 27.86 ± 1.22°C temperature during April 2002 as compared to August 2002 (average maximum 30.55 ± 1.21° C and minimum 26.04 ± 0.61° C) to common carp fry.

Hardness of water had a significant influence in reducing the toxicity of deltamethrin (Table 3). At lower temperature, *i.e.*, in August 2002, LC_{50} value in very hard water (T5) was significantly higher (Table 2) than the LC_{50} value in soft water (T4). At higher temperature, *i.e.*, in April 2002, LC_{50} value in very hard water (T2) was also significantly higher when compared with the LC_{50} value in soft water (T1). LC_{50}, $LC_{0.1}$ and SAR values in these four conditions are mentioned above and are also given in Table 2 and Fig. 2. Reduced toxicity of deltamethrin in very hard water may be due to any one of these two reasons or both. As the hardness of water increases, concentrations of $C1^{-1}$; SO_4^{2-}, CO_3^{2-} and HCO_3^- salts of calcium and magnesium increase. This may cause lower solubility of deltamethrin due to the salting out effect and thus, lower the toxicity. Another reason may be the high level of calcium in very hard water, which is reported to protect the gills of fish from the damage by the pollutant (Chapman *et al.*, 1998). The lowered toxicity of deltamethrin in very hard water might also be due to the change in the chemistry of biotic receptor sites with the increase in hardness, which reduces the permeability of deltamethrin.

Sediment was able to reduce the acute toxicity of deltamethrin significantly (Table 3) in comparison to respective water treatments. LC_{50}, $LC_{0.1}$ and SAR values of very hard water and soil sediment

treatments (T3 and T6) and very hard water treatments (T2 and T5) are mentioned before (Table 2). This might be due to the adsorption or complexation reaction. The soil used for the experiment contained 24.88% clay, 21% silt and 3.01% organic matter as also 3 g well decomposed powdered humus which was added. This 3 g added humus acted as a source of both POC and DOC in the test solution, which were estimated to be 22 ± 2.0 and 7.5 ± 1.5 mg l^{-1}, respectively. Under aerobic condition, clay colloids, FeOOH, MnOOH and POC are the dominant binding phases in sediments (Tessier *et al.*, 1996). Deltamethrin is lipophilic (fat soluble) or hydrophobic in nature and therefore, adsorbed on the surfaces of the colloidal sediment (clay/humus, FeOOH/MnOOH) and POC (Muir *et al.*, 1994). DOC also formed stable complexes with hydrophobic organic compounds in this case with deltamethrin, thus decreasing the bioavailability of deltamethrin to common carp fry (Day, 1991). Exposure time could not produce any significant impact up to 96 hours on the acute toxicity of deltamethrin to common carp fry (Table 3).

From the above discussion, it is clear that deltamethrin is very toxic to fish in soft water (96 LC_{50}: 0.102 µg l^{-1}) without sediment at high ambient temperature. When the hardness factor was taken into account, acute toxicity reduced almost five times (0.495 µg l^{-1}). When sediment was included with hardness, acute toxicity reduced 17.7 times (1.808 µg l^{-1}). When influence of water was taken into account along with water hardness and sediment factors, total reduction in the acute toxicity of deltamethrin at lower temperature was 79 times (8.073 µg l^{-1}). In terms of $LC_{0.1}$, the reduction in toxicity from T1 to T6 was 125.54 times. So, relation of water hardness and soil sediment with lethal concentration is positive or direct, while with temperature, it is negative or inverse. It can be concluded that in the natural field conditions, deltamethrin may not be as toxic as emphasized by many laboratory toxicity data. Increased hardness of water, reduced air temperature and various sediment factors (*e.g.*, POC, DOC, amount and nature of soil colloids, etc.) can protect fish from acute toxicity of deltamethrin under natural conditions and therefore, must be taken into account for correct quantification of toxicological effects. During winter months in the natural aquatic ecosystems where water is hard in nature and high colloidal sediment is observed (*e.g.*, in sewage-fed aquaculture or in lentic water body the bottom of which is clayey and decomposed organic matter is present), acute toxicity of deltamethrin may be less as compared to other aquatic systems.

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