



Temperature changes alter the acute toxicity responses of cypermethrin in Zebrafish

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Abstract

The study was carried out to determine the effect of temperature changes on acute toxicity of pyrethroid pesticide cypermethrin in zebrafish. A two-day renewal bioassay system for 96h was conducted to find out LC₅₀ value of cypermethrin at two temperature regimes i.e. 25°C and 30°C considering as T₁ and T₂, respectively. During the determination of LC₅₀ in both temperatures, blood glucose (mg/dL) levels were measured at lower concentration (0.25 µg/L) of cypermethrin. The results of acute toxicity test at 96h LC₅₀ values were calculated through probit analysis. It was found that 96h LC₅₀ for T₁ and T₂ groups were about 2.1 and 1.4 µg/L, respectively. Significantly lower LC₅₀ of cypermethrin at T₂ compared to T₁ showed that higher temperature increased the toxicity of cypermethrin. There was a significant increase ($P < 0.05$) in blood glucose level (mg/dL) in 0.25 µg/L compared to 0 µg/L concentration of cypermethrin at both treatments. Dissolved oxygen decreased and free CO₂ increased significantly ($P < 0.05$) with increasing temperature, while the pH of the water was almost unchanged throughout the study period. The present study indicated the impact of increased temperature on pesticide toxicity in the aquatic ecosystem.

Key words: Temperature, acute toxicity, cypermethrin, blood glucose, zebrafish

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Introduction

Pesticides are one of the notorious causes of environmental pollution because the use of pesticides is increasing day by day as part of new High Yielding Varieties (Uddin *et al.*, 2016). The Bangladeshi government has promoted the use of pesticides to increase agricultural yields (Dasgupta *et al.*, 2007). The application of pesticides may lead to contamination of the aquatic environment through several ways including spray drift, runoff, and leaching (Van den Brink, 2013; Shahjahan *et al.*, 2017a). The insecticides used in the agricultural fields are generally classified into four major groups: Organochlorines, Carbamates, Pyrethroids and Organophosphates. The test chemical, cypermethrin belongs to the pyrethroids group and is

extremely toxic to fish. Durability of its action in water is about 7-30 days. Pesticides can directly affect fish through alteration in normal behavior (Satyavardhan, 2013; Ullah *et al.*, 2015), physiological functions (changes in hematological parameters), and histo-architectural changes in liver, kidney, intestine etc. (Ahmed *et al.*, 2015; Salam *et al.*, 2015; Sharmin *et al.*, 2015 & 2016; Hossain *et al.*, 2016).

Fish are very susceptible to physical and chemical changes of water which may be reflected in their blood components (Wilson and Taylor, 1993). The survival, distribution, reproduction and normal metabolism of fish depend on aquatic environmental temperature

(Shahjahan *et al.*, 2017b) and inability of fish to adapt for temperature fluctuations may cause in death because of changes in metabolic pathways (Forghally *et al.*, 1973). The normal range of water temperature in the tropics to which fish are adapted is 25-35°C (Howerton, 2001). Although it varies from species to species, temperature can increase to a point that may become harmful for growth and damage physiological processes (Portner *et al.*, 2010).

The zebrafish, *Danio rerio* (Hamilton), is one of the most common vertebrate model organisms in genetics, neurophysiology, biomedicine and the developmental biology (Amsterdam and Hopkins, 2006; Shahjahan *et al.*, 2013). As a hardy, attractive, and most active aquarium fish, the zebrafish is easily recognized by its distinctive horizontal stripes. Blue-purple horizontal stripes run from gill to tail, setting off the slim compressed silver-gold body of this attractive fish. Harmful effects of cypermethrin are reported in various teleosts, such as damage of gill and brain in case of *Tor putitora* (Ullah *et al.*, 2015), shortness of breath in common carp (Yücel and Özkul *et al.*, 2016) and flabby and degenerative ovarian follicles along with several behavioral abnormalities in freshwater fish *Channa striatus* (Mondol *et al.*, 2015). Considering all the points the present investigation was conducted to elucidate the effects of temperature on acute toxicity of cypermethrin in zebrafish, *Danio rerio*.

Materials and Methods

Experimental fish: Healthy and active specimens of zebrafish, *Danio rerio* with a length of 3.0 ± 1.0 cm and body weight 1.0 ± 0.78 g were collected from different ponds around the faculty building of Fisheries, Bangladesh Agricultural University. The fish were maintained in aquaria of 20L capacity at $25 \pm 0.5^\circ\text{C}$ and the medium of aquaria renewed daily which is performed under a controlled natural photo-regimen (14/10 h, light/dark) for a period of 21 days with adequate aeration facility before the experiments. The fish were fed twice a day.

Test chemicals: Commercial grade pesticide cypermethrin 10EC was collected from Mymensingh town and used as the test chemical to conduct the experiment and for the determination of LC₅₀ of cypermethrin.

Experimental design and determination of LC₅₀: The present experiment was conducted at two different temperature regimes i.e. 25°C and 30°C considering as treatment one (T₁) and treatment two (T₂), respectively. The required temperature was maintained by using thermostat (REI-SEA, Japan, 300 WATTS) and adequate aeration was maintained throughout the experimental period. To acclimatize the fish to high temperature, temperature was gradually increased ($\Delta 1^\circ\text{C}$ per 12h) from normal temperature (25°C) to the target temperature conditions (30°C). A two-day renewal bioassay system for zebrafish was used to determine the LC₅₀ value of cypermethrin at defined temperature conditions, following exposure of 96h according to the standard method of American Public Health Association (APHA, 2005). Ten fish were transferred into each aquarium used to find out the range for the ultimate test. Each treatment of the experiment was performed in triplicate with five test concentrations (100 L) of narrow range and a control was also maintained as given below:

T₁ (25°C): 0.25, 0.50, 1, 2, 4 and 8 µg/L cypermethrin along with a control (without cypermethrin).

T₂ (30°C): 0.25, 0.50, 1, 2, 4 and 8 µg/L cypermethrin along with a control (without cypermethrin).

Mortality was assessed at 24, 48, 72, and 96h after reached the desired temperature conditions and dead fishes were removed immediately. Several behavioral changes, such as reduced activity, equilibrium imbalance, abnormal swimming and motion inactivity of the fish were observed during the exposure period.

Measurement of blood glucose: During the determination of LC₅₀ in both temperature conditions, blood glucose (mg/dL) levels were measured at lower concentration (0.25 µg/L) of cypermethrin. Blood was collected from the caudal peduncle and immediately

analyzed for the estimation of glucose (mg/dL). Blood glucose (mg/dL) was measured by EasyMate® GHb, bloodglucose/hemoglobin dual-function monitoring system using glucose strips.

Water quality parameters: Some water quality parameters such as dissolved oxygen (mg/L), free CO₂ (mg/L) and pH were measured during the experimental period. Dissolved oxygen (mg/L) was measured by a DO meter (Model DO5509, Lutron, Taiwan) and pH by a portable pH meter (Model RI 02895, HANNA Instruments Co.). The free CO₂ (mg/L) was measured using phenolphthalein indicator and 0.0227N NaOH titrant.

Statistical analysis: Values were presented as mean \pm standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to assess statistically significant differences among the different temperature and different sampling days. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using SPSS Version 14.0 for Windows (SPSS Inc., Chicago, IL).

Results

Effect of temperature on acute toxicity of cypermethrin: The 96h LC₅₀ values of cypermethrin were found to be 2.1 $\mu\text{g/L}$ and 1.4 $\mu\text{g/L}$ in T₁ (25°C) and T₂ (30°C), respectively (Figure 1). The 96h LC₅₀ values of cypermethrin at T₂ (30°C) was found to be significantly ($P < 0.05$) lower compared with calculated LC₅₀ values of cypermethrin at T₁ (25°C). The linear transformation of percentage mortality and log concentration of cypermethrin are showed in Figure 2a & b.

Effects of temperature on blood glucose levels: The values of blood glucose levels are presented in Figure 3. Blood glucose levels (mg/dL) were significantly ($P < 0.05$) increased in pesticide treated fish in both temperature at both 48 and 96h exposures. On the other hand, blood glucose levels were significantly

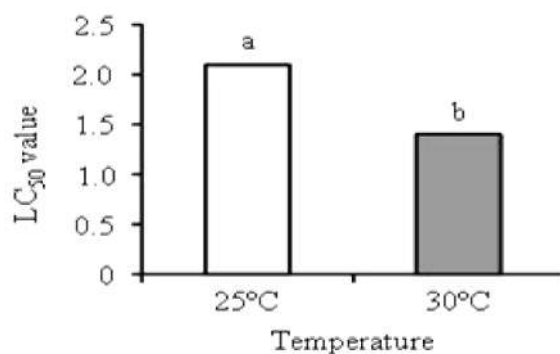


Figure 1. LC₅₀ values of cypermethrin at two temperature conditions (25°C & 30°C). Values are expressed as mean and bar bearing different superscripts vary significantly ($P < 0.05$).

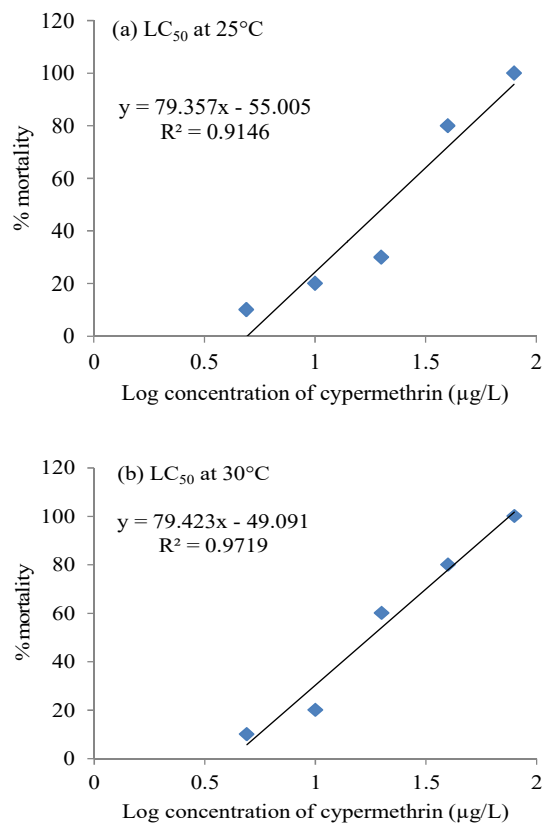


Figure 2. Graph showing linear transformation and the relationship of probit of log concentration of cypermethrin used to determine LC₅₀ at (a) 25°C and (b) 30°C.

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increased at T₂ (30°C) compared to T₁ (25°C) in control and pesticide treated groups at 48h exposure period.

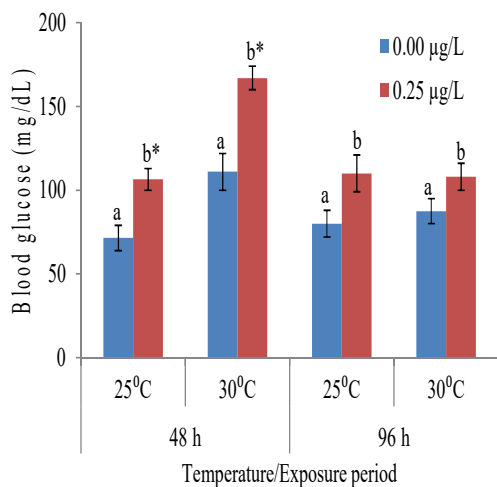


Figure 3. Effects of cypermethrin on blood glucose levels (Mean \pm SD) in zebrafish. Values accompanied by different letters are statistically significantly different ($P < 0.05$) between fish treated without pesticide and with pesticide. Asterisk indicated significantly different ($P < 0.05$) between temperature treatments.

Water quality parameters: Dissolved oxygen (mg/L) values were found to be decreased significantly ($P < 0.05$) with increasing pesticide concentration in both temperature treated groups (25°C and 30°C), and free CO₂ (mg/L) values were found to be increased with increasing pesticide concentration. On the other hand, the value of pH was remained almost unchanged throughout the study period irrespective of the temperature conditions as well as with and without pesticide exposure (Figure 4).

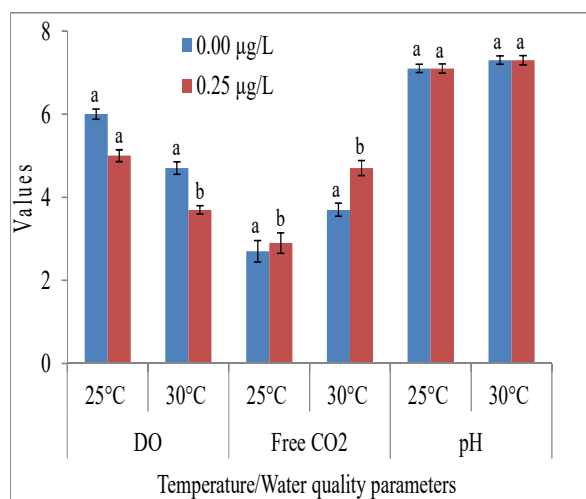


Figure 4. Water quality parameters. Values are expressed as Mean \pm SD. Mean value in bar bearing different superscripts varies significantly ($P < 0.05$).

Discussion

In the present study, the zebrafish were subjected to cypermethrin at different concentrations at two temperature regimes (25°C and 30°C) for 96h. The 96h LC₅₀ of cypermethrin at 30°C was found to be significantly low when compared with estimated LC₅₀ for cypermethrin 25°C. Low LC₅₀ of cypermethrin with an increase in temperature showed that at a higher temperature, cypermethrin toxicity increased and adversely affected the fish protective responses. However, the exact mechanism of decreased toxicity is not illustrated. Abnormal behavior of experimental fish such as gasping frequently with high respiration rate was observed at higher temperature when exposed to cypermethrin. Further, at higher temperature, dissolved oxygen decreases, resulting in increased respiratory rate which tends to increase the absorption rate of cypermethrin and hence, more concentration of cypermethrin is needed to diminish the host fish detoxification activity, resulting in higher fish mortality at a lower concentration. Temperature can affect the amount of toxicant accumulated and its toxicity by adversely affecting respiration and uptake rate,

biotransformation and excretion rates (McIntyre, 1998). Although, evidences are available with regard to the effect of temperature on cypermethrin toxicity, it deals with reference to fish species. To quantify the influence of selected temperature regime and cypermethrin exposure on water quality, various physico-chemical parameters of water were estimated in terms of values and the significant differences among the treatment groups. The effect of temperature on dissolved oxygen and free carbon dioxide were observed and found to be significantly ($P < 0.05$) different in control group (T_1), when compared with T_2 . The test animals showed restlessness, frequent surfacing, loss of balance and irregular opercula movement thus gradually becoming lethargic followed by their mortality. These observations were in accordance to the study of (Chattopadhyay et al., 2006) who reported certain erratic behavioral patterns of fish during the exposure period to herbicides. The fish exhibited restlessness and peculiar tumbling motion before death. The degree of toxicity produced by the poisonous substance is dose dependent upon environmental conditions such as temperature, pH, oxygen content and presence of residual molecules (Capkin et al., 2006). It is well known that protein, carbohydrate and lipid play a major role as energy precursors in fish under stress conditions. Enzymes play a significant role in metabolism and detoxification of pollutants and as such pesticides can produce metabolic changes at cellular level by way of influencing enzyme systems. Thus reduction in dissolved oxygen in water can become vital as unavailability of oxygen to the cells tends to accumulate more toxic intermediate metabolites (Meister, 1992). Increase in temperature beyond the optimum range (temperature tolerance) of the host fish adversely affects its enzyme system, causing low resistance to pollutant resulting in higher mortality.

Blood glucose has been shown to be a sensitive indicator of environmental stress. In the present study, increased glucose levels in the fish exposed to high temperature might be due to the mobilization of

glycogen into glucose to meet the increased demand for energy used in combating the stress induced on the fish by high temperature. Glucocorticoids and catecholamine hormones are known to produce hyperglycemia in animals and stress stimuli elicit rapid secretion of these hormones from adrenal tissue of the fish (Pickering, 1981). Such increase may be due to enhanced gluconeogenesis response of stressed fish in their attempt to satisfy their new energy demands (Winkaler et al., 2007). As the respiratory metabolism is being depressed, stored intracellular glycogen is utilized under such condition; the hyperglycemic hormone is released for the degradation of glycogen and glucose thus leaked into the blood causing hyperglycemia (Hossain et al., 2015). Ahmed et al., (2016) reported that the blood glucose levels were significantly ($P < 0.05$) increased in all concentrations (0.5, 1.0 and 2.0 mg/L) of sumithion compared to control (0 mg/L) in zebrafish. In the present investigation, high temperature exposure resulted in a significant increase in plasma glucose concentration without any mortality, demonstrating the response of exposed fish to metabolic stress.

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