

The Effects of Pesticide on the Developmental Zebrafish (*Danio rerio*) Embryos

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Abstract: *Paclobutrazol (PBZ) is a pesticide which is widely used as a plant growth regulator and fungicide in agriculture by farmers. Periodic contamination with agricultural pesticides potentially impairs the structure and or the function of stream biota, and agricultural streams are recognized as some of the most impacted on earth. Application of the chemical has contaminated the soil and water, however the biological toxicity of PBZ was less investigated. Observation on living embryos showed that PBZ exposure has significant affect survival rate and also higher induced pericardiac edema at 24 hpf, 36 hpf and 48 hpf in treated embryos as compared to control embryos. This research shows that PBZ can severely damage zebrafish embryonic development at early time PBZ exposure.*

1. Introduction

The use of bioengineered plants for food production, including plants engineered to express plant-incorporated protectants, has markedly increased over the past decade (EPA, 2007). Rasmussen *et al.* (2012) report about periodic contamination with agricultural pesticides potentially impairs the structure and or the function of stream biota, and agricultural streams are recognized as some of the most impacted on earth.. Indeed, the co-occurrence of numerous pesticides in agricultural streams has been reported frequently in scientific studies and monitoring programs.

Soil treatment (1 mg/l) and foliar treatment (25 mg/l) with PBZ improves the photosynthetic activity and water balance of tomato cv. Precador. PBZ treatment accelerates fruit formation and increases early fruit yield. PBZ treatment of plants at the time of pricking out ensures the production of fruits without any residual retardant which are the harmless to human health (Berova and Zlatev, 2000). The aim of study are to evaluate the critical stages exposure times PBZ on developmental zebrafish (*Danio rerio*) embryos.

2. Materials and Method

Fish maintenance, embryos treated and collection

Use zebrafish (*Danio rerio*) for animal model, paclobutrazol (PBZ), dimethyl sulfoxide (DMSO) for control. Zebrafish (*Danio rerio*) adults and embryos were maintained at 28 °C under standard conditions. Zebrafish embryos (N=100 per plate) were start to expose use DMSO and PBZ at different stages embryos development (24 hpf, 36 hpf, 48 hpf, 60 hpf, 72 hpf and 96 hpf). Embryos were expose to 100 ppb, 1 ppm, and 5 ppm PBZ in RO water.

Survival rate and Pericardiac Edema of embryos

Zebrafish embryos (N=100 per plate) were exposed to different concentrations of PBZ (100 ppb, 1 ppm and 5 ppm) also control containing 0,02% DMSO. During the assay, the plate were placed in the incubator with the temperature 28 °C. The die embryos were recorded and removed daily during 5 dpf.

Alcian Blue Staining

Alcian blue staining was performed to investigate the pharyngeal arch development and neurocranium. After embryos were fixed with 4% PFA, and then embryos were washed with 1 ml PBST 3 times for 10 minute each time. Embryos were bleached in 1.5 ml of 10% H₂O₂ and two drops of 2 M KOH at room temperature for 2 hours. After bleached, embryos were washed with 1 ml PBST 3 times for 5 minutes each time. Then embryos were incubated in Alcian blue staining solution (0.1% Alcian blue, 70% ethanol, 1% HCl) 5 hours at room temperature. After staining, embryos were destained by using fresh acidic ethanol (70% ethanol, 5% HCl) for overnight in room temperature (wash the embryo by acidic ethanol until the embryo body was became transparent). Finally the embryos were kept in 80% glycerol and stored at 4 °C. The staining result was imaged using Olympus microscope imaging systems.

Statistic analysis

Data were presented as the mean ± standard deviation. Significant differences in survival rate

data between the treatment groups were evaluated using Least Significant Different (LSD) methods to calculate the multifactorial ($P < 0.05$). All statistical analyses were performed using R language.

3. Results and Discussion

PBZ exposure caused survival rate in zebrafish embryos

Zebrafish (*Danio rerio*) has become a popular model to study vertebrate development. Adult zebrafish breed all year long, they can be maintained easily and their embryos are transparent. (Tiso N., et al., 2009). Survival rate PBZ treated embryos at 24 hpf (hours post fertilization), 36 hpf (hours post fertilization) and 48 hpf (hours post fertilization) have significantly decrease and significantly at 5 ppm PBZ treated embryos (see Table 1).

Table 1. Percentage of survival rates of Zebrafish Embryos

Stages of Zebrafish Developmental	Percentages of Survival Rate (%)	
24 hpf	DMSO	96.67±1.25
	100 ppb	94.33±1.69
	1 ppm	92.67±1.69
	5 ppm	87±3.08
36 hpf	DMSO	97±1.41
	100 ppb	96±0.82
	1 ppm	94±1.41
	5 ppm	88±2.55
48 hpf	DMSO	97.67±0.47
	100 ppb	97.33±0.47
	1 ppm	95.67±0.94
	5 ppm	91±2.45
60 hpf	DMSO	97.67±0.47
	100 ppb	97.33±0.47
	1 ppm	96.67±0.94
	5 ppm	95±0.41
72 hpf	DMSO	97.67±0.94
	100 ppb	97±0
	1 ppm	96.67±0.94
	5 ppm	95.67±0.47
96 hpf	DMSO	98±0.82
	100 ppb	97.33±0.47
	1 ppm	97.33±1.24
	5 ppm	95.67±1.47

Furthermore, McKim (1977) reported at the conclusion that in at least 80 % of the cases long-term toxicity could be predicted by results from studies with early life-stages.

PBZ exposure induced pericardiac edema in zebrafish embryos

The embryos showed several abnormalities especially pericardiac edema after PBZ exposure. The percentages of pericardiac edema was different in PBZ treated embryos groups at 24 hpf (hours post fertilization), 36 hpf (hours post fertilization) with 5 ppm concentration compare with control (see Figure 1). The data showed that the higher pericardiac edema was 77.37±2.05% in 5 ppm PBZ treated groups embryos at 24 hpf (hours post fertilization). The lower pericardiac edema was 2.79±0.47% in 5 ppm PBZ treated embryos groups at 24 hpf (hours post fertilization) (see Table 2).

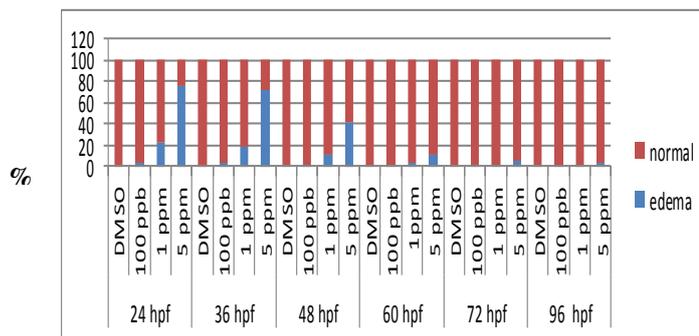
Table 2. The percentages of pericardiac edema of Zebrafish Embryos

Stages of Zebrafish Developmental	Percentages of Pericardiac Edema (%)	
24 hpf	DMSO	0±0
	100 ppb	4.24±0.82
	1 ppm	21.94±2.87
	5 ppm	77.37±2.05
36 hpf	DMSO	0±0
	100 ppb	3.47±1.25
	1 ppm	18.79±2.05
	5 ppm	73.55±2.36
48 hpf	DMSO	0±0
	100 ppb	1.37±0.47
	1 ppm	11.85±2.05
	5 ppm	39.33±2.62
60 hpf	DMSO	0±0
	100 ppb	0.68±0.94
	1 ppm	3.78±0.94
	5 ppm	10.17±0.94
72 hpf	DMSO	0±0
	100 ppb	0±0
	1 ppm	2.41±0.94
	5 ppm	5.23±0.82
96 hpf	DMSO	0±0
	100 ppb	0±0
	1 ppm	2.39±0.94
	5 ppm	2.79±0.47

Edema can accompany cardiovascular dysfunction caused by interconnection between the osmoregulatory function of the skin and pronephric kidney and also the circulatory function of the heart and vasculature (Carney, S. A., et al., 2005). PBZ can induce the oxidative stress (Ding et al, 2008) with increases the production of highly free radical and oxidizing species (Anbazhagan, M. and Chellapan, P. R., 2009).

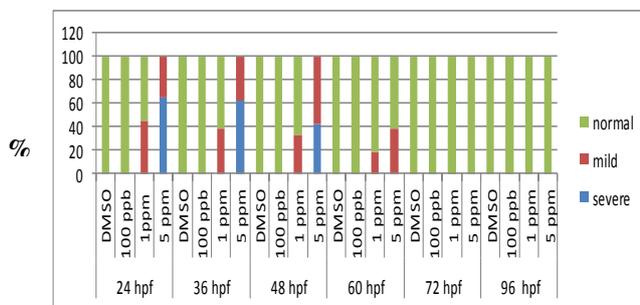
PBZ disrupts the head skeleton formation

Development of head skeleton was analyzed by alcian blue stained and classified to normal, mild and severe. The percentage of malformation defective head skeleton of PBZ treated embryos at early stages development was higher compared with the control groups (see Figure 2). Detrich, H. W., *et al.*, (2009) displayed that the skeletons of vertebrates are remarkably similar. Centuries of studies of bone morphology and the fact that bones are preserved as fossils has revealed that identical bones form much of the skull, vertebrae, and appendicular skeleton in fish, amphibians, reptiles, and mammals.



Different stages Zebrafish embryos development

Figure 1. Average percentage of pericardiac edema of zebrafish embryos



Different stages Zebrafish embryos development

Figure 2. Percentage of head skeleton development malformation

4. Conclusion

Taken together, our results indicates PBZ exposure at early developmental stages of zebrafish embryos were affected percentages of survival rates, pericardiac edema and head skeleton formation.

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