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Research Article

TOXIC EFFECTS OF DELTAMETHRIN ON EARLY LIFE STAGES OF ZEBRAFISH *DANIO RERIO*

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ABSTRACT

Deltamethrin is a synthetic pyrethroid pesticide that kills insects through dermal contact and digestion. It is applied for a range of commercial crops and recreational uses, and by extension controls a variety of pests. Synthetic pyrethroids, such as deltamethrin, are toxic substances that lead to generation of reactive oxygen species, which harm living organisms. The present study was carried out to know the effect of commercial product of deltamethrin, decis on parameter involving percentage of mortality during development in zebrafish embryos, *Danio rerio.* Studies shows increased mortality with increase in concentration and time. Our work illustrates that usefulness of zebrafish as a model to probe and identify the windows sensitive to toxicants, in our case decis.

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INTRODUCTION

Deltamethrin is a synthetic insecticide based structurally on natural pyrethrins, which rapidly paralyze the insect nervous system giving a quick knockdown effect. Deltamethrin has a rapidly disabling effect on feeding insects and for this reason there is hope that it may be useful to control the vectors of "nonpersistent" viruses (viruses that can be passed on by the vector within a few minutes of starting to feed on the plant). Deltamethrin's mode of action is thought to be mainly central in action, or at least originate in higher nerve centers of the brain. Although pyrethroids are often considered to be "safer" pesticides because of their low to moderate acute toxicity to nontarget species, their increased use raises concerns of potential adverse effects, particularly in sensitive populations such as children. This concern is intensified by recent studies indicating that children are exposed to pyrethroids during development. For example, pyrethroid metabolites have been found in the urine of pregnant women (Berkowitz *et al.*, 2003; Whyatt *et al.*, 2002). A recent study also found that 67% of a cohort of preschool children had detectable levels of the pyrethroid metabolite 3-phenoxybenzoic acid in their urine (Morgan *et al.*, 2007). Lu *et al.* (2006, 2009) have also found pyrethroid metabolites in urine of elementary-age children that appear to be primarily the result of residential exposure.

METHODS AND MATERIALS

Maintenance of Parental Fish

Wild type adult Zebrafish (*Danio rerio*) used in this study were bred in our aquarium facility for two generations. Females and males are kept in a ratio of 2:1 in an aquaria filled with filtered tap water with the oxygen saturation of more than 80% and P^H at 7.0 ± 0.3. The water temperature was maintained at 26 ± 1^0C at a 14h: 10h day and light regime. Fish were regularly provided with varied diet comprising of freshly hatched live brine shrimp (*Artemia nauplii*) once a day, supplemented with vitamin fed dried flake food twice a day. The aquarium water was aerated continuously with stone diffusers connected to mechanical air compressor. Renewal of water is done in a semi-static manner and the aquaria screens were cleaned daily. The excess amount of food and fecal matter was removed from the water and healthy environment was provided before experimentation. The water quality and cleanliness of aquaria was monitored regularly and reset to initial state. Less than 1% of the population died during acclimatization.

Zebrafish egg Collection

Embryos were collected from breeding stock of healthy, unexposed mature male and female zebrafish which were above the six months. Care was taken such that the fish were

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free of macroscopically discernable symptoms of infection and disease. The spawning glass trays were covered with a fine nylon net with an appropriate mesh size for eggs to fall through were placed in the aquaria on the evening before the spawning was required. Plant imitations made of plastic serving as spawning substrate are fastened to the nylon mesh. The fish were left undisturbed over night. Eggs were spawned synchronously at dawn of the next morning. After the light was turned on the next morning embryos were generated by natural mating and then collected within 30 minutes after spawning. Newly fertilized eggs were collected from the spawning trays and embryos were rinsed several times with tap water and their quality was checked under the microscope being sure to select the healthy fertilized eggs for the experiment. Unfertilized eggs were identified by their milky color and discarded. The dead embryos appear white because of the coagulation of precipitation of proteins.

Preparation of Test Solution

Decis EC 11% (W/W) manufactured by Bayer's company was purchased from local Agro-Chemical stores. Using the formula $C_1V_1=C_2V_2$, the concentration of deltamethrin present in the decis was calculated. Then the stock solution was prepared by dissolving 1.9ml of decis in distilled water and made it upto 100ml standard flask.

Embryo-Larval Toxicity Test

Embryos at the same developmental stage (4hpf) were collected and rinsed with tap water. Exposure experiments were carried out by placing 100 embryos in 500ml of water containing glass chambers with different concentrations of decis, 0.2, 0.4, 0.6, 0.8, 1.0µg/l of decis and unexposed embryos were used as controls. Exposure experiments were carried out in triplicate. Morphological and developmental endpoints were observed under an optic microscope in control and treated embryos at 24, 48, 72 and 96hpf. The toxicant was added everyday to maintain exact concentration of decis and the water quality. Occasional stirring as well as replacement of the medium were done daily to ensure even distribution of the chemical. Embryos and larvae were daily observed under a stereomicroscope (Magnus MLX) and magnification used for observation was 4X and 10X for eggs and larvae. Embryos and larvae were considered dead when no heart beat was observed and dead embryos or larvae were removed immediately and at each observation time mortality was recorded every 24h.

RESULTS

At 24hpf No mortality was observed among embryos in the control and all treated groups exposed to 0.2, 0.4, 0.6, 0.8 and 1µg/l of commercial grade deltamethrin, decis.

At 48hpf There was no mortality in the control group. In the treated groups the mortality rates were 0%, 1%, 2%, 2% and 4% in the embryos exposed to 0.2, 0.4, 0.6, 0.8 and 1µg/l, respectively to commercial grade deltamethrin, decis.

At 72hpf There was 1% mortality in the control group. In the treated groups the mortality rates were 1%, 13%, 18%, 30% and 45%. in the embryos exposed to 0.2, 0.4, 0.6, 0.8 and 1µg/l, respectively to commercial grade deltamethrin, decis.

At 96 hpf All the fertilized eggs were hatched when observations were made at 96hpf. One percent mortality was observed in the control group also.

The percentage of mortality observed at 0.2 and 0.4µg/l of decis was 10% and 20% respectively, whereas the percentage of mortality observed at 0.6, 0.8 and 1µg/l of decis were 33%, 55% and 70% respectively.

Percent Mortality Observed at different time periods after exposing fertilized eggs to different concentrations of commercial grade deltamethrin, decis

S.No	Observations Made at	Control	Concentration of deltamethrin added				
			0.2 µg/l	0.4 µg/l	0.6 µg/l	0.8 µg/l	1.0 µg/l
1	24 hpf	0	0	0	0	0	0
2	48 hpf	0	0	2	2	2	2
3	72 hpf	1	1	13	18	30	45
4	96 hpf	1	10	20	33	55	70

DISCUSSION

At 96hpf, survival of larvae treated with the highest concentration of decis was reduced, suggesting that the zebrafish developmental toxicity of decis got stronger with exposure duration. Exposure to the COOH-functionalized MWCNT test solutions to the zebrafish embryos caused the death of 35 to 45%, slightly increasing with the increasing of the concentration. The vast majority of the deaths occurred at 96 hpf (Olasagasti *et.al*., 2008). Ahmad and Ansari (2011) observed that the mortality of Azacel, a neem based pesticide treated zebrafish embryos at lowest concentration (0.02µg/l) was 8.67% and highest concentration (0.10µg/l) it increased to 89.33%. colloidal silver (cAg) produces almost 100% mortality at 120hpf, while cAu (gold nanoparticles) produces less than 3% mortality (Ilan *et.al*., 2009), at Perfluorooctanesulfonate (PFOS) concentrations of 0.1 and 0.5 mg/L, no significant increase in mortality was observed over the whole exposure time, where as a significant increase in mortality was observed at PFOS concentrations of 1, 3 and 5mg/L.

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