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Research Article

EFFECT OF SYNTHETIC PYRETHROID, DELTAMETHRIN CAUSING DELAY IN HATCHING OF ZEBRAFISH EMBRYOS

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ABSTRACT

Water is the primary life giving resource and is fundamental to human way of life. Its availability is an essential component in socio-economic development. Today a number of significant factors have an impact both on this resource and on managing water in integrated, sustainable and equitable manners. Pyrethroids act very quickly to produce symptoms of lost coordination and paralysis which are known as "the knockdown effect", and which are often accompanied by spasms and tremors that induce intense repetitive activation in sense organs and in myelinated nerve fibers. Deltamethrin is not mobile in the environment because of its strong adsorption on particles, its insolubility in water, and very low rates of application; However, it still presents risks to the ecosystem in which it is applied. Experiment was carried out on zebrafish embryos exposing to different concentrations of deltamethrin. The present study reveals that time of hatchability increases with increasing concentrations.

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. 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.

Maintenance of Parental Fish

Wild type adult Zebrafish (*Danio rerio*) used in this study was 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^{0}C 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 free of macroscopically discernable symptoms of infection and disease. The spawning glass trays were covered with a fine

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nylon net with an appropriate mesh size for eggs to fall through was 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 upto 72hpf and unexposed embryos were used as controls. Exposure experiments were carried out in triplicate. Percentages of hatchlings 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 of hatching was recorded every 24hours. From 48hpf embryos started to come out of the chorion asynchronously and the data of hatchings were recorded.

RESULTS

At 48hpf

The percentage of hatchlings in the control were 26%, while the percentage of hatchlings in the different concentrations of decis 0.2µg/l, 0.4µg/l, 0.6µg/l, 0.8 µg/l and 1µg/l were 24%, 20%,18%,18% and 11% respectively. The unhatched eggs were still alive.

At 72hpf

The percentage of embryos coming out of their chorions in the control and 0.2µg/l, decis treated were 100%, whereas the hatching process is prolonged with a reduced percentage of hatchlings at higher concentrations in the treated groups of 0.4µg/l, 0.6µg/l, 0.8 µg/l and 1µg/l of decis were 79%, 63%, 58% and 43%.

At 96hpf

As earlier stated the exposure of decis induced concentration dependent exposure with the highly reduced hatchlings at 96hpf when compared to control and 0.2µg/l where the 100% hatchlings were observed. Fertilized eggs exposed to 0.4 µg/l, 0.6µg/l, 0.8µg/l and 1µg/l were 98%, 90%, 90% and 67% respectively. However, the eggs remained unhatched at 96hpf did not hatch later and they died.

DISCUSSION

The morphology of the hatching gland at both the cellular and intracellular levels was disorganized. The hatching gland releases enzymes that break down the chorion and allow embryos to hatch. Delayed hatching and embryonic aestivation are strategies employed to prolong the residence of an embryo within the egg in response to adverse environmental condition. Delayed hatching allow protection of young during unfavourable conditions and also permit synchronization of hatching when environmental surroundings promote optimum survival.

Unfortunately, the enzyme activity of the hatching enzyme which exists transitorily is easily affected by environmental materials. The structure and the function of the protease might be destroyed by toxicants and might block pore canals of the chorions, resulting in the shortage of oxygen supply to the development of embryos (Fan and Shi, 2002).

One reason for the delay or failure of hatching may be the developmental abnormalities, which may partially or completely limit the ability of developing embryos or larvae to break outer chorion and hatch out. The other reason may be the inhibition of enzymes involved in hatching Chorion hardening may be responsible for the effects of toxicants on hatching and some pollutants may act directly on the HGCs and on secretion of the enzymes that these cells produce. The failure to hatch may be related to behavioral deficits, specifically, the animals appear to have delayed and weakened spontaneous motion (Haendel *et.al*.2004).

The present work is in accordance with the work of George and Nagel (2000) who demonstrated the exposure of deltamethrin on zebrafish embryos resulted in decrease in hatching success. The high percentage mortality of hatching success was obtained for control group (91.3%). The development of larvae was influenced by deltamethrin. Hatchability of embryos was reduced in a dramatic way at 0.80µg/L. During embryonic development the embryo is protected by the egg shell, and the metal concentration in the egg is relatively low. The shell breaks at the beginning of hatching and ceases to shield the embryo.

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