ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 12(D), pp. 30033-30035, December, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

ABNORMAL BEHAVIORAL RESPONSE OF ZEBRAFISH EMBRYOS ON EXPOSURE TO DELTAMETHRIN

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DOI: http://dx.doi.org/10.24327/ijrsr.2018.0912.2981

ARTICLE INFO

Article History:

Received 6th September, 2018
Received in revised form 15th October, 2018
Accepted 12th October, 2018
Published online 28th December, 2018

Key Words:

Pyrethroid, pesticide, deltamethrin, zebrafish, *Danio rerio.*

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. In the present study larvae of zebrafish exposed to 0.4µg/l and higher concentrations of decis showed a less sensitive to touch, reduced movement and in some case paralysis (abnormal touch response) indicating neurotoxicity. Behavioral abnormalities associated with neurotoxicity that resulted from decis include spastic behavior at the lower concentrations and reduced motility and paralysis at the highest concentration.

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.

MATERIALS AND METHODS

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^{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

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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 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.

Experimental Design

Fertiised eggs of zebrafish were exposed to different concentrations of decis, at 4hpf, 24hpf, 48hpf, 72hpf and transferred to normal water at 96hpf and behavioral responses were observed in the hatched larvae.

RESULTS

Touch Response

The touch response reflects the integration of mechanosensory input from Rohon-Beard neurons in the spinal cord and motor output via the hindbrain. The response to a mechanical stimulus (touch) was used as a measure of sensorimotor integration (Saint-Amant and Drapeau, 1998). Hatched fish were gently touched on the head with a probe. Animals that swam away after one, two stimuli were scored as responders. All others were scored as nonresponders. Unexposed fish showed a normal touch response at 48, 72 and 96hpf as indicated by effective swimming movements of the tail

The natural reflex of the larval is to escape when touched, a commonly listed sensory response. Control larval were able to sense and react to the probe, even before being touched whereas doses of 0.4ug/l and higher elicited no movements, even when fish were touched multiple times. Impaired motor response was dose dependent and the prevalence of larvae with an abnormal touch response was significantly higher in fish exposed to > 0.6 ug/l decis.

Swimming behavior

A similar lack of response in higher decis treatments was noted in the behavior study. Larvae at doses of 0.8 and 1ug/l exhibited an inability to swim or move. The few larvae that did show motility at treatments of 0.4 and 0.6 ug/l exhibited tail paralysis (defined as incomplete lack of movement) and relied on pectoral fin movements and body convulsions to produce subtle motions. Less general activity loss of equilibrium, remaining motionless on the aquarium bottom. Larvae in the control group were alert to tapping on the bottom of the culture plate and showed avoidance behavior instantly, while the decis treated ones showed slow or no responses to stimuli.

Fish in the exposure groups were transferred to clean system water. All of the larvae in 0.2 and 0.4ug/l concentration. regained touch response after few days. By contrast only few regained normal response at a concentrations of 0.6 and 0.8ug/l and at highest concentration none of the surviving larvae regained touch response. Spontaneous trunk contractions at 24 hpf were noted in all exposure groups. However, the frequency was lower in the exposed animals than in the control.

DISCUSSION

Behavior of zebrafish larvae were observed when exposed to different toxicants at different stages. Results from locomotor assays showed that zebrafish larvae of 96 h exhibited impaired swimming behavior after exposure to 50, 100, and 200μgL^{-1} of bifenthrin from 3 to 84 hpf (Jin *et.al*, 2009). Sodium benzoate treatment caused significant reduction of locomotor activity ($p<0.001$), in treated zebrafish embryos when compared with the control (Chen *et.al*, 2011). Exposure of zebrafish embryos to $25mgL^{-1}$ dichlorvos dose caused significant slowing of swimming activity on day 6 and 9 after fertilization (Sisman *et.al*., 2010) and multi- and single-day exposures to methylmercury (MeHg) 15µg/L caused reduced swimming activity and prey capture ability. Further continuous embryonic exposure of 10µg/L of the same chemical significantly reduced spontaneous swimming activity (Samson *et.al*., 2001). A significant decrease in the distance swum by larvae (5 and 10 dpf) was observed when exposed to silver (Powers *et.al*., 2010).

Tiedeken *et.al*, (2005) observed the primary behavioral response in all domoic acid (5 dpf) treated zebrafish embryos indicated as rapid and constant pectoral fin movements. Further embryos treated with 12.6 and 4.0 mg/kg DA exhibited a hyperactive pectoral fin movement, even when still in chorion and treatment with (12.6 and 16.8mg/kg) exhibited hyperactive pectoral fin behavior while the rest of the body elicited no movements. Larvae at doses of 1.2mg/kg and higher exhibited an inability to swim or move, exhibited tail paralysis and relied on pectoral fin movements and body convulsions to produce subtle motions.

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How to cite this article:

Nalini D and Philip G.H.2018, Abnormal Behavioral Response of Zebrafish Embryos on Exposure to Deltamethrin. *Int J Recent Sci Res.* 9(12), pp. 30033-30035. DOI: http://dx.doi.org/10.24327/ijrsr.2018.0912.2981

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