Research Paper | ZOOLOGY | E-ISSN : 2455-295X | Volume : 2 | Issue : 7 | July 2016

DECREASED VIABILITY IN ZEBRAFISH EMBRYOS ON EXPOSURE TO DECIS

Dr.D.NALINI[1]

[1] LECTURER IN ZOOLOGY, THE AAS COLLEGE,ADONI.

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 the viability decreases with increasing concentrations.

Key words: Pyrethroid, viability,zebrafish.

Introduction

Low to middle income developing regions as well as highly developed countries will face water stress in the future, unless existing water reserves are managed effectively. Discharge of untreated wastewater is leading to increased pollution and depletion of clean water resources. Runoff from agricultural fields contains pesticides and fertilizers that pollute surface water and groundwater. The UN estimates that the amount of wastewater produced annually is about 1,500 km^3, six times more water than exists in all the rivers of the world (UN WWAP 2003). Worldwide, infectious diseases such as waterborne diseases are the number one killer of children under five years old and more people die from unsafe water annually than from all forms of violence, including war (WHO 2002). By 2025, India, China and selected countries in Europe and Africa will face water scarcity if adequate and sustainable water management initiatives are not implemented.

Loss of biodiversity is an important indicator of lowered resilience and the current deterioration in fresh water bodies (greater than either marine or terrestrial) is of great concern (UNWWDR March 2006). Globally, 24 percent of mammals and 12 percent of birds connected to inland waters are considered threatened (UN WWAP 2003). In some regions, more than 50% of native freshwater fish species are at risk of extinction, and nearly one- third of the world's amphibians are at risk of extinction (Vié et.al., 2009). Freshwater species face an estimated extinction rate five times greater than that of terrestrial species (Ricciardi and Rasmussen 1999). Even drinking water quality in developed countries is not assured. In France, drinking water testing uncovered that 3 million people were drinking water whose quality did not meet WHO standards, and 97% of groundwater samples did not meet standards for nitrate in the same study (UN WWAP 2009).

Review of Literature

The term "pyrethrum" refers to the dried and powdered flower heads of a white-flowered, daisy-like plant belonging to the Chrysanthemum genus. Pyrethrum's insecticidal properties were recognized in the middle of the 19th century when Caucuses tribes used it for the control of body lice (Jones 2001). The

superior insecticidal properties of *C. cinerariaefolium* were first discovered around 1845 and is currently cultivated in the USA, Japan, Kenya, Brazil, the Democratic Republic of the Congo, Uganda and India (Bhat et.al.,1995). After the discovery of the constituents of pyrethrins, researchers searched for derivatives of pyrethrins that had a higher resistance to photodegradation. This search directly led to the synthesis of pyrethroids.

Synthetic pyrethroids are synthesized derivatives of naturally occurring pyrethrins, which are taken from pyrethrum, the oleoresin extract of dried chrysanthemum flowers. The insecticidal properties of pyrethrins are derived from ketoalcholic esters of chrysanthemic and pyrethroic acids. These acids are lipophilic and readily penetrate many insects and paralyze their nervous system (Reigart et.al., 1999). The advantages of pyrethrins and pyrethroids are that they are highly lipophilic, have a short half-life in the environment, have low toxicity to terrestrial vertebrates and do not biomagnify like older chemical classes, such as organochlorines, pyrethroids are readily taken up by biological membranes and tissues. The majority of pyrethroids were derived by modifying the chrysanthemic acid moiety of pyrethrin I and esterifying the alcohols. Low toxicity in mammals is attributed to two factors: limited absorption of some pyrethroids; and rapid biodegradation by mammalian liver enzymes (ester hydrolysis and oxidation). Insects without this liver function exhibit greater susceptibility to these chemicals. Synthetic pyrethroids have been developed in order to improve the specificity and activity of pyrethrins, while maintaining the high knockdown and low terrestrial vertebrate toxicity.

Varieties of xenobiotics have differential effects on various fish, which include activation of detoxification, metabolic and genetic pathways. Fish are endowed with defensive mechanisms to counteract the impact of reactive oxygen species (ROS) resulting from metabolism of various chemicals or xenobiotics. Oxidative stress develops when there is an imbalance between pro-oxidants and antioxidants ratio, leading to the generation of ROS. Environmental contaminants such as herbicides, heavy metals and insecticides are known to modulate antioxidant defensive systems causing oxidative damage in aquatic organisms by ROS production (Risso-de Facerney et al., (2001); Achuba and Osakwe, (2003); Liu et al.,(2006); Monteiro et al.,(2006). ROS such as hydrogen peroxide (H_2O_2), superoxide anion O_2 and hydroxyl radical at supranormal levels can react with biological macromolecules potentially leading to enzyme inactivation, lipid peroxidation (LPO), DNA damage and even cell death (Pena-Llopis et al.,2003; Banudevi et al.,2006) but at low concentrations their effects are less pronounced.

Embryos of zebrafish also exhibited stress indicating effects of xenobiotics at early life stages. Wu et.al., (2011) reported that zebrafish embryos after a short-term exposure to various concentrations of Bisphenol A (BPA), nonylphenol (NP), and their mixture (BPA-NP) for 4h postfertilization (hpf) to 168h found to enhance the production of hydroxyl radicals and lipid peroxidation in a concentration-dependent manner. The content of total glutathione (TG), reduced glutathione (GSH), and oxidized glutathione (GSSH), as well as the activity of antioxidant enzymes including catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase were all significantly inhibited indicating the occurrence of oxidative stress. After exposure to trichlorfon for 96h on zebrafish embryos showed increased activity of glutathione-S-transferase, inhibition of cholinesterase, lactate dehydrogenase and catalase activity (Coehlo et.al., 2011). Inhibition of cholinesterases and carboxy lesterases were noticed in zebrafish embryo by insecticide aldicarb and its metabolite aldicarb- sulfoxide (Kuster et. al., 2007).

MAINTENANCE OF PARENTAL FISH

Wild type adult Zebrafish (*Danio rerio*) used in this study were bred in our aquarium facility for two gen-

erations. 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±11^{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 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.

BIOLOGY OF ZEBRAFISH

The Zebrafish, (*Danio rerio*), a small tropical fish native to the rivers of India and South Asia (Eaton and Farley 1974) has emerged to be one of the best described and most popular vertebrate model species (Coverdale et.al., 2004, Nagel 2002,) in many scientific fields including ecotoxicology.

It commonly inhabits streams, canals, ponds and slow moving to stagnous water bodies. The fish is named so because of five uniform pigmented horizontal blue stripes on the side of the body. Its shape is fusiform and laterally compressed with its mouth directed upwards. Females have a larger whitish belly. The Zebrafish can grow to 6.4 cms and its life span is around 2-3yrs, which may extend to 5yrs sometimes.

Zebrafish is omnivorus and is primarily eats zoo plankton, insects and insect larvae and phyto plankton. Most *Danios* accept common food flakes and tubifex worms in the aquarium. They thrive best at water temperature 22-28°c. The approximate generation time for the *Danio* is 3-4 months. Fertilization is external. Fertilized eggs almost immediately become transparent, a characteristic that makes *Danio rerio* a convenient research model species.

ADVANTAGES OF ZEBRAFISH

i) As per OECD guidelines for analyzing the influence of chemical, zebrafish was recommended as a model species representing aquatic vertebrate (OECD 1998 , 2000).

ii)Well understood, easily observable and testable development behavior.

iii)Rapid embryonic development.

iv)Large and transparent embryos that develop outside mother.

v)Drugs may be administrated by adding directly to the tank.

vi)Unfertilized eggs turns milky white, so we can separate them easily.

vii)Demonstrated similarity to mammalian models and humans in toxicity testing.

viii)Zebrafish shares the same set of genes and have similar target sites for treating human diseases.

MATERIALS AND METHODS

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.

Viability Test

Experiment was conducted after exposing the fish for 6 days with 2µg/L and 4µg/L of deltamethrin. Controls were maintained. Eggs were collected every day, 6 days before the addition of toxicant and for 6 days during the exposure time. Fertilized and unfertilized eggs were separated, counted and recorded. Viability of fertilized eggs were observed. Everyday water is renewened and toxicant is added. Controls were maintained. The experiment will be done in triplicates.

ZEBRAFISH EGG COLLECTION

Embryos were collected from breeding stock of control and exposed 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 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.

Result

S.No	Observations Made at	Percentage of viable embryos			
		24hrs	**48hrs**	**72hrs**	**96hrs**
1	**control**	0	24	74	99
2	**2 µg/l**	0	18	62	90
3	**4 µg/l**	0	10	41	62

The percentage of embryonic movements within the chorions in the control were 99%, whereas the hatching process is prolonged with a reduced percentage of hatchlings at higher concentrations in the treated groups when compared with the controls.

Discussion

Emodin caused developmental arrest at the shield

stage, which indicated that emodin might disturb the origination of the hatching enzyme, chorionase (He et.al., 2012)

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 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.

Ishibashi et.al., (2004) reported hatching delay in embryos of O,latipes exposed to 0.31mg/l of triclosan. Exposure of zebrafish embryos to dispersed SWCNTs agglomerates in the aquatic environment delayed hatching but did not influence embryonic development and survival of exposed embryos (Cheng et.al., 2007). Because the size of the pores on the embryo chorion was nanoscaled and the size of the SWCNTs agglomerates was microscaled, the authors suggested that the chorion of zebrafish embryos was an effective barrier for protection from SWCNTs agglomerates. Embryo exposure to chitosan nanoparticles and ZnO nanoparticles resulted in a decreased hatching rate (Hu et.al.,2011). At higher concentration of pesticides the eggs of *Cyprinus carpio communis* Linn.died before hatching because the pesticide affects the activity of hatching enzymes (Kaur and Toor, 1977). According to Dave and Xiu (1991) low concentrations of copper (0.25 µg/L), lead (30 µg/L), mercury (0.2µg/L) and nickel (80 µg/L) can interfere in hatching and survival of the zebrafish. The hatching rates were 44% in the water control and 0% when zebrafish embryos exposed to nanomaterials((nC60) at 60hpf (Zhu et.al.2007). Shi et.al.,(2008) reported that over 90% of the control zebrafish embryos hatched out of the chorion between 48 and 72 hpf. At 72 hpf, there was a significant dose-dependent decrease in the hatching rate of the PFOS-treated groups compared with the hatching rate of the control group. The embryos of the control group developed normally in embryo medium, and hatching began at 48 hpf and was completed at 72 hpf. The hatching rates of embryos treated with 1.0-µM or higher concentrations of celastrol were significantly lower than that of the control. The median effect concentration (EC50) for delayed hatching was 1.02µM (Wang et.al., 2010). Witeska et al. (1995) reported delayed hatching of Cyprinus carpio incubated at cadmium exposure. Common carp embryos exposed to copper or lead tended to hatch longer compared to the controls (Slomin´ska 1998).

The effect of metals during hatching may be related to increased metal penetration into the egg. 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. A sudden increase of metal concentration in the egg probably causes stress in the embryos, resulting in premature hatching of underdeveloped larvae or mortality of larvae (Jezierska et.al., 2009).

REFERENCES

[1] Achuba F.I., Osakwe S.A., 2003. Petroleum-induced free radical toxicity in African Catfish (Clarias gariepinus). Fish Physiol. Biochem., 29: 97-103.

[2] Banudevi S., Krishnamoorthy G., Venkataraman P., Vignesh C., Aruldhas M.M., Arunakaran J., 2006. Role of tocopherol on antioxidant status in liver, lung and kidney of PCP exposed male albino rats. Food Chem. Toxicol., 44: 2040-2046

[3] Dave, G. and Xiu, R., 1991. Toxicity of mercury, copper, nickel, lead and cobalt to embryos and larvae of zebrafish, Brachydanio rerio. Arch. Environ. Cotan. Toxicol. 21: 126-134.

[4] Kuster E., Altenburger R., 2007. Suborganismic and organismic effects of aldicarb and its metabolite aldicarb-sulfoxide to the zebrafish embryo (Danio rerio). Chemosphere. 68(4): 751-760.

[5] Monteiro D.A., Almeida J.A., Rantin F.T., Kalinin A.L., 2006. Oxidative stress biomarkers in the

freshwater characid fish Brycon cephalus, exposed to organophosphon insecticide Folisuper 600 (Methyl parathion). Comp. Biochem. Physiol., 143: 141-149.

[6] Nagel, R. (2002). DarT: The embryo test with the zebrafish, Danio rerio—a general model in ecotoxicology and toxicology.ALTEX 19(Suppl l):38–48.

[7] Pena-Llopis S., Ferrando M.D., Pena J.B., 2003. Fish tolerance to organophosphate- induced oxidative stress is dependent on the glutathione metabolism and enhanced by N- acetylcysteine. Aquatic Toxicol., 65: 337-360.

[8] Risso-de Facerney C., Devaux A., Lafaurie M., Girard J.P., Bailly B., Rahmani R., 2001. Cadmium induces apoptosis and genotoxicity in rainbow trout hepatocytes through generation of reactive oxygen species. Aquatic. Toxicol., 53: 65-76.

[9] Witeska M, Jezierska B, Chaber J (1995) The influence of cadmium on common carp embryos and larvae. Aquaculture 129:129–132.