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Effect of glyphosate-based herbicide on early life stages of Java medaka (*Oryzias javanicus*): A potential tropical test fish

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ABSTRACT

Glyphosate is globally a widely used herbicide, yet there is little information on their toxicity to marine fishes. Java medaka, a small tropical fish native to coastal areas in several Southeast Asian countries, is viewed as a suitable candidate for toxicity test and thus was used for this study. Java medaka adults were cultured in the laboratory and the fertilized eggs of the F_2 generation were exposed to different concentrations of glyphosate-based herbicide (100, 200, 300, 400 and 500 ppm) until they hatched. The survival and hatching rates of the embryos, changes in the heart rate and morphological impairments were recorded. Generally, survival and hatching percentage decreased as glyphosate concentration increased. Absence of pectoral fin(s) and cornea, permanently bent tail, irregular shaped abdomen, and cell disruption in the fin, head and abdomen are among the common teratogenic effects observed. Furthermore, risk factor also increased with the increased in glyphosate concentrations.

1. Introduction

Glyphosate (*N*-(phosphonomethyl)glycine) is a broad-spectrum, systemic, post-emergence organophosphorus herbicide which has been widely used around the globe since its introduction in the market in the 1970s. Firstly formulated under the brand name Roundup®, this herbicide is effective in killing a wide variety of terrestrial and aquatic weeds. It is commonly used for agriculture, horticulture and silviculture as well as garden maintenance. Glyphosate is highly water soluble, considered a non-persistent molecule in soils but relatively persistent in water (PPDB, 2012). Glyphosate is very slightly or moderately toxic to the aquatic animals (Giesy et al., 2000; Solomon and Thompson, 2003), but the surfactant polyoxyethylene amine (POEA) added in the commercial formulation is considered more toxic (Brausch and Smith, 2007; Tsui and Chu, 2003, 2004). The combination of the two is yet more toxic.

Glyphosate is highly polar and water-soluble. Due to these physical–chemical properties, analysis of water samples is rather difficult and time-consuming. So glyphosate is not a common parameter in water quality control. Therefore, only few analysis data about the occurrence in the aquatic environment are known (Smith et al., 1996). In Malaysia glyphosate is one of the major weed control used in oil palm plantation and other major crops. Due to high rainfall in the country, glyphosate will end up in the river and ultimately into coastal areas.

The medaka fish belong to a diverse group of small fish distributed in large areas in Asia, occupying the freshwater, brackish water and saltwater. They are important model species among bony fish. The most established laboratory fish among the medaka fish is the Japanese medaka (*Oryzias latipes*) which is distributed in the freshwater of Japan, Korea and China (Naruse et al., 1993; Naruse, 1996). One native species in Peninsula Malaysia, Singapore, Indonesia, Thailand and Western Borneo (Iwamatsu et al., 1982; Magtoon and Termvidchakorn, 2009; Roberts, 1998), the Java medaka, (*O. javanicus* (Bleeker, 1854)) has been used in several studies to represent the marine fish. Java medaka is widely distributed in the coastal areas of Peninsular Malaysia and they are available all year round. It has been utilized in several ecotoxicological studies (Imai et al., 2005, 2007; Ismail and Yusof, 2011; Koyama et al., 2006; Woo et al., 2006; Yu et al., 2006). In this study, we used the early life stages of the fish to investigate their responses after exposure to glyphosate-based herbicide. The early life stages of fish have long been recognized as very sensitive biological material (Marchetti, 1965). A number of studies have reported the relevance of utilizing early life stages of fish for assessing the ecological risk posed by pollutants in the aquatic environments (Eaton et al., 1978; Ismail and Yusof, 2011; Shazili and Pascoe, 1986; Strmac et al., 2002; Ward et al., 1982; Wadekind et al., 2007). To date, there is no report on the response of Java medaka to glyphosate exposure.

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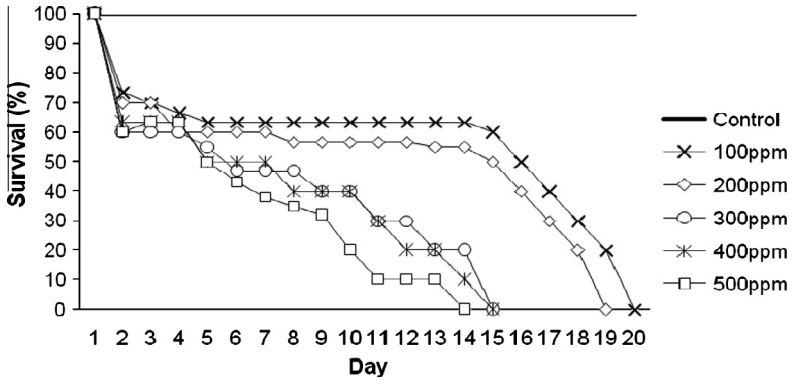

Fig. 1. Survival rate of early life stages of Java medaka after exposure to different concentrations of glyphosate.

2. Materials and methods

Java medaka was cultured in the Department of Biology, Universiti Putra Malaysia. Culture conditions were as follows: salinity 20 ppt, temperature 28–30 °C (ambient), pH 5.5–6.5, D.O 6.0–8.0 mg L^{-1}, and photoperiod 14 h light:10 h dark. Newly-spawned Java medaka eggs clusters from F_2 generation were collected gently from the female's body by hand. The clusters of eggs were separated using forceps and then surface sterilized using a mixture of NaCl, KCl, $CaCl{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$ and methylene blue for 5 min (Kinoshita et al., 2010). The eggs were then incubated in separate test solutions containing notional concentrations of 100, 200, 300, 400 and 500 ppm glyphosate (Roundup®). Roundup® (Monsanto) was purchased from a garden center. The formulation contains 360,000 ppm glyphosate. Maximum dose of glyphosate as recommended by the manufacturer is 500 ppm. Thus, in this study we used this concentration and several concentrations lower.

Stock solutions of 1000 ppm were prepared in distilled water, from which small aliquots were added to saline water in order to obtain desired concentration. The saline water was made using artificial sea salt with salinity 20.0 ppt. The final salinity of the test solution is ensured by dissolving small amounts of artificial sea salt in it till it reached 20.0 ppt as measured using multiprobe meter (YSI 556). This salinity was chosen based on the average salinity of the localities of the fish we investigated during our survey on their habitat (Yusof et al., 2012). Control is a saline water of 20.0 ppt.

Ninety fertilized eggs were exposed to each glyphosate concentration. Exposure was done under semi-static system where the test solutions were renewed every 24 h. Observations on the development of the embryos were made every 24 h using stereomicroscope. Death and hatching of embryos were recorded. The heart rate of each living embryos was counted. Any developmental impairment was recorded and captured. Exposure was done till three days post-hatch. Hatched embryos were transferred to saline water of 20.0 ppt for further observation. Differences between treatments were compared using Kruskal Wallis one-way analysis of variance.

3. Results and discussion

Fig. 1 shows the survival rate of Java medaka embryos after exposure to different concentrations of glyphosate within 20 days. Different concentrations of glyphosate used significantly affected the survival rate ($p < 0.05$). Generally, survival rate reduced as the glyphosate concentration increased. Embryos exposed to 300, 400 and 500 ppm glyphosate were all dead by the end of the exposure period. As the survival of the embryos decreased, the hatching rate also decreased when exposure concentrations increased (Fig. 2). Rank of hatchability based on glyphosate concentrations is as follows: control > 100 ppm = 200 ppm > 300 ppm > 400 ppm = 500 ppm. Glyphosate was found to inhibit the enzyme 5-enolpyruvyl shikimic acid-3-phosphate synthase (EPSPS) which halts the production of chorismate. It results in the cessation of aromatic amino acid synthesis, which in turn reduces protein synthesis and growth that will cause cellular disruption and death to organism (Vera et al., 2010). Only 50% of the embryos exposed to 100 ppm glyphosate died after 16 days of exposure. Love et al. (2011) reported that the increase in chemical loadings in water will lead to negative effects on aquatic ecosystems such as fish kills. This previous study is comparable to this study where increase in glyphosate concentration resulted an increase in death of embryo. Many of the embryos were weakened by glyphosate exposure and do not survive hatching which is an energy consuming process (Jezierska et al., 2009).

Change in the heart rates of the embryos under different glyphosate concentrations is shown in Fig. 3. The heart of the embryo started to beat at day-three post-fertilization. Kawasaki et al. (2008) reported the normal heart rate for Japanese medaka ranged from 106 to 113 beats per minute for 3 day observation. Normal heart rate for early life stages of Java medaka found in this study for 16 day observation was between 101 and 140 beats per minute. Exposure to glyphosate initially increased the heart beat compared to normal and it later on fluctuated and finally slowed down or halted. The highest heart rate was found in embryos exposed to 500 ppm glyphosate on the fifth day of exposure (162 beats per minute). Increased heart beat shows that the embryos are under stress. Fluctuations of heart rate suggest the adaptations to changing environment whereby the thickness of the heart was still able to withstand it (Kawasaki et al., 2008). As glyphosate causes cellular disruption (Vera et al., 2010) to the heart wall, less pumping was produced.

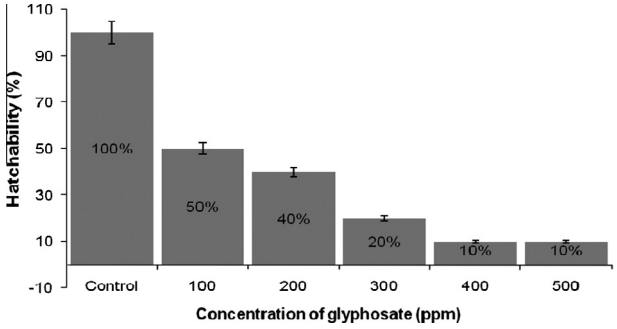

Fig. 2. Percentage of hatchability of Java medaka embryos in different concentrations of glyphosate. Vertical bar represents standard error.

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