Research Article

# Acute Toxicity of Paraquat and Glyphosate on embryonic development of Climbing Perch (*Anabas testudineus*)

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**Abstract**

This study examined the toxicity of paraquat and glyphosate on *Anabas testudineus* embryos. The embryos were treated with paraquat at 0.01, 0.03 and 0.1 mg/L, and glyphosate at 0.02, 0.05 and 0.2 mg/L. The effects of these substances were examined by monitoring the three endpoints: mortality, hatching rate and heart rate. The results showed that all concentrations of paraquat and glyphosate affected the fish embryos with increased mortality and decreased hatching rate. As compared to the control, there were no significant differences in the head and tail bud stage, somite stage, and heart rate, whereas mortality, and hatching rate showed significant difference ($p<0.05$). The results revealed that paraquat was more toxic than glyphosate.

**Keywords**: Paraquat, Glyphosate and Climbing perch embryos

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# พิษเฉียบพลันของพาราควอทและไกลโฟเสทต่อระยะคัพภะของปลาหมอไทย (*Anabas testudineus*)

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**บทคัดย่อ**

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อทดสอบความเป็นพิษเฉียบพลันของพาราควอทและไกลโฟเสทต่อการพัฒนาระยะคัพภะของปลาหมอไทย โดยนำไข่ของปลาหมอไทยมาทดสอบกับสารทั้ง 2 ชนิด โดยใช้ความเข้มข้นของพาราควอทที่ 0.01 0.03 และ 0.1 mg/L และความเข้มข้นของไกลโฟเสทที่ 0.02 0.05 และ 0.2 mg/L ตามลำดับ จากนั้นสังเกตอัตราการตาย อัตราการฟัก และอัตราการเต้นของหัวใจ ผลการทดลองพบว่า ไข่ปลาหมอไทยที่ได้ทดสอบกับพาราควอทและไกลโฟเสทที่ทุกระดับความเข้มข้นมีผลต่ออัตราการตายและอัตราการฟัก ส่วนอัตราการเต้นของหัวใจ และการพัฒนาด้านอื่นๆ เช่น ระยะการสร้างส่วนหัวและหาง และระยะสร้างกระดูกสันหลัง พบว่าไข่ปลาหมอไทยในกลุ่มทดลองมีการพัฒนาใกล้เคียงกับกลุ่มควบคุม และเมื่อเปรียบเทียบความเป็นพิษของพาราควอทและไกลโฟเสท พบว่า พาราควอทมีผลต่ออัตราการตายของไข่ปลาหมอไทยมากกว่าไกลโฟเสท นอกจากนั้นผลการวิเคราะห์ทางสถิติพบว่า อัตราการตายและอัตราการฟักมีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p<0.05$)

**คำสำคัญ**: พาราควอท, ไกลโฟเสท และ คัพภะปลาหมอไทย

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## Introduction

Thailand uses large quantities of pesticides to control crop pests, vectors of disease, and thereby increase yields. Recently, a marked increase in the use of more powerful agricultural chemicals has boosted agricultural production and efficiency. The Office of Agriculture Regulation of the Department of Agriculture reported that about 70,000 tons of pesticides comprising 265 individual active ingredients were imported into Thailand in 2010. The most abundant herbicides were glyphosate and paraquat (OAE, 2010).

Paraquat dichloride is one of the most widely used herbicides in the world. It is also toxic to humans when swallowed (Ogamba *et al*., 2011). Paraquat is classified as a moderately toxic chemical for lethal toxicity, with a relatively low potential to bio-accumulate in the aquatic environment. However, many studies have shown that paraquat can damage important tissues in aquatic animals (Arunlertarce and Kawatsu, 1992; Omitoyin *et al*., 2006; Babatunde *et al*., 2014; Deivasigamani, 2015). The pesticides are washed into the aquatic environment through run-off and leaching and negatively impact on the flora and fauna. According to the United States Environmental Protection Agency (USEPA, 2007), herbicides contain an active ingredient which is highly toxic to both cold and warm water fishes.

Glyphosate is a non-selective herbicide that degrades rapidly in soil and natural water, with DT 50 values ranging from 3-14 days. Glyphosate suppresses plant growth through interference with the production of essential aromatic amino acids by inhibiting the enzyme enolpyruvylshikimate-3-phosphate (EPSP) synthase. This enzyme is responsible for the biosynthesis of chorismate, an intermediate in phenylalanine, tyrosine, and tryptophan biosynthesis (Pedron *et al*., 2006). The acute toxicity of glyphosate is considered to be low by the WHO (WHO, 1994). However, commercial glyphosate formulations are more acutely toxic than glyphosate (Peixoto, 2005). Glyphosate also affects energy metabolism, free radical processes, acetylcholineesterase activity (Langiano *et al*., 2008), and immune responses of histological changes in hepatocytes of *Oreochromis niloticus* (Szarek *et al*., 2000), and *Cyprinus carpio* (Jiraungkoorskul *et al*., 2003).

Fish are widely used to evaluate the health of aquatic ecosystems and physiological changes serve as biomarkers of environmental pollution (Kock *et al*., 1996). The fish *Anabas testudineus* was selected as the test organism because of its euryhaline and eurythermal nature and unique position in the food chain. They are quite sturdy and ideally suited for experimentation in the laboratory over longer time periods. These fish are cultured in ponds and have a very good commercial value with high nutrition and good taste (Afsar, 2012). Therefore, this study evaluated the effects of paraquat and glyphosate on the embryonic development of *A. testudineus*.

## Materials and Methods

**Test organisms**

Adult climbing perch, *A. testudineus* strain used for egg production originated from the Maha Sarakham Inland Fisheries Research and Development Center, Maha Sarakham, Thailand. The fish were maintained in a cement tank with a flow-through system at ambient temperature. They were fed twice a day (08.00 a.m. and 04.00 p.m.) with commercial fish food. The physicochemical characterization of the water used for fish bioassay was carried out using standard methods (APHA, 1998) and the following

values were obtained: temperature 27ºC, pH 6.20-7.20, dissolved oxygen 5.30-6.20 mg/L, alkalinity 51.25-136 mg/L, and water hardness 144-185 mg/L.

**Exposure conditions**

The effects of pesticides were investigated by exposing developing *A. testudineus* embryos inside their egg to a range of paraquat dichloride (Gramoxone®) (0, 0.01, 0.03 and 0.10 mg/L) and glyphosate–isopropyl ammonium (Roundup®) concentrations (0, 0.02, 0.05 and 0.20 mg/L). Solutions were prepared with double-distilled water. The range of tested concentrations were based on application rate of both pesticides. For the test with paraquat and glyphosate, glass Petri dishes were used and the stock solutions were kept in glass bottles.

**Embryo toxicity test**

The eggs were collected and rinsed several times with double distilled water, and then transferred to various exposure chambers. At around 2-4 h post fertilization, the fertilized eggs (blastula stage) were selected and transferred to glass Petri dishes (10 per plate) containing different concentrations of paraquat and glyphosate. Replacement of the mediums was performed daily to ensure an even distribution of the chemical. The experiment was conducted with a total of 90 eggs per treatment group.

The development of blastula eggs was monitored at specified time points (t = 2-4, 6, 8, 12, and 24 h). Observation was then extended until the time of hatching for the different exposure groups. Endpoints were used to assess the effects of paraquat and glyphosate regarding egg and embryo mortality, gastrulation, somite formation, movement, tail detachment, pigmentation, heart rate (number of contraction of the heart per min: bpm) and circulation, and hatching success (Fig. 1). Malformations and inhibitory tendencies were also noted and described among juveniles from both control and treatment groups using a stereomicroscope.

**Statistical analysis**

Treatment effects on the developmental parameters were determined using one-way analysis of variance (ANOVA), followed by Duncan's new multiple range test to compare the treatment groups with their respective control. Significant difference occurred for a given parameter when $p<0.05$.

## Results

**Paraquat treatment**

At the highest paraquat concentration (0.10 mg/L), the mortality was 100% at 2 h of exposure time, whereas embryos exposed to 0.01 and 0.03 mg/L paraquat showed significant mortality rates of 8.89% and 44.44%, respectively (Table 1). For all treatments, paraquat significantly decreased the hatching success rate compared to the control group (Table 2). The average heart rate was unaffected by the test concentrations and controls with a tendency for reduction at higher paraquat concentrations of 0.01 and 0.03 mg/L.

**Glyphosate treatment**

At 2 h to 12 h, the mortality of embryos treated with concentrations of 0.02 mg/L glyphosate or higher showed a trend to increase. This finding, however, was significant only at the highest concentration tested (0.20 mg/L) (Table 1).

**Table 1** Mortality rate (%) in *A. testudineus* embryos exposed to paraquat and glyphosate

| Tested Substances (mg/L) | | Mortality (%) | | | |
|---|---|---|---|---|---|
| | | 2 h | 4 h | 8 h | 12 h |
| Paraquat | 0 | 0±0[a] | 1.11±3.33[a] | 1.11±3.33[a] | 1.11±3.33[a] |
| | 0.01 | 8.89±12.7[b] | 20±18.71[b] | 27.78±16.41[b] | 31.11±12.69[b] |
| | 0.03 | 44.44±15.09[c] | 86.67±12.25[c] | 92.22±8.33[c] | 94.44±7.26[c] |
| | 0.10 | 100±0[c] | 100±0[c] | 100±0[c] | 100±0[c] |
| Glyphosate | 0 | 0±0[a] | 1.11±3.33[a] | 1.11±3.33[a] | 1.11±3.33[a] |
| | 0.02 | 1.11±3.33[a] | 7.78±6.67[a] | 8.89±7.82[a] | 10±7.07[a] |
| | 0.04 | 3.33±5[a] | 10±7.07[a] | 11.11±7.82[a] | 11.11±7.82[a] |
| | 0.20 | 11.11±10.54[b] | 21.11±10.54[b] | 33.33±11.18[b] | 38.89±15.37[b] |

Note: Groups with different letters *are* significantly different ($p < 0.05$)

At 12 h of exposure, 98% of all fish had hatched in the control. Embryos exposed to 0.2 ml/L glyphosate showed a significantly reduced hatching rate at 12 h with mean of 61% (Table 2). The average heart rate was unaffected by the test at nominal concentrations and controls.

In addition, no significant malformations were observed in *A. testudineus* embryos exposed to the different treatment groups. Minimal abnormalities as edema and eye and tail defects were found in both control and treated embryos at<5% (Fig.2). However, the results were not significant.

**Table 2** Hatching success rate (%) in *A. testudineus* embryos exposed to paraquat and glyphosate.

| Tested Substances (mg/L) | | Hatching success rate (%) |
|---|---|---|
| Paraquat | 0 | 98.89±3.33[a] |
| | 0.01 | 68.89±12.69[b] |
| | 0.02 | 5.56±7.26[c] |
| | 0.10 | 0±0[c] |
| Glyphosate | 0 | 98.89±3.33[a] |
| | 0.02 | 90±7.07[a] |
| | 0.04 | 88.89±7.82[a] |
| | 0.20 | 61.11±15.37[b] |

Note: Groups with different letters are significantly different ($p<0.05$)

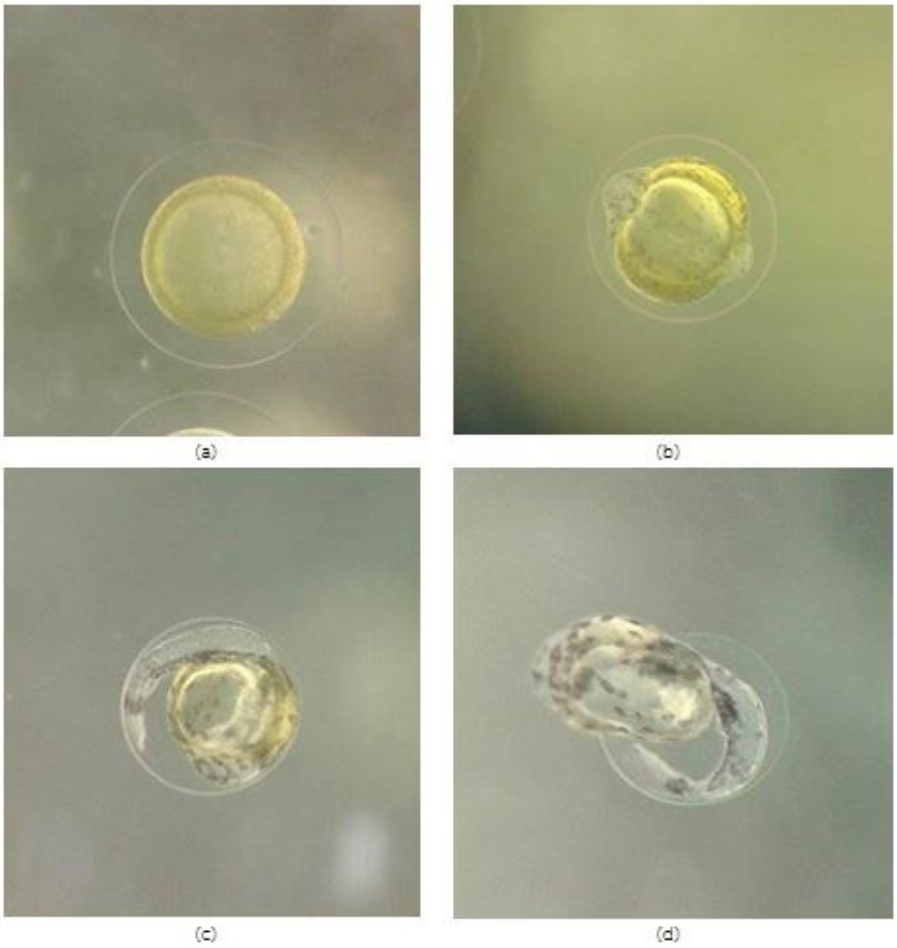


**Fig. 1** *A. testudineus* embryonic development at different stages after fertilization (a) gastrula (5 h); (b) head and tail bud (7 h); (c) somite (8 h); (d) hatching (12 h)

## Discussion

Our study clearly showed that paraquat is dramatically increased mortality rates and reduced embryonic development in *A. testudineus.* Glyphosate at 0.20 mg/L induced adverse effects in the fish embryos. Several types of fish have been determined as sensitive to paraquat. Ogamba *et al*. (2011) noted that juvenile American catfish (*Clarias gariepinus*) exposed to paraquat dichloride at 0.4 mg/L showed significant decrease in all values of metabolites in the muscles and gills. Other studies on fish toxicity testing reported a sensitivity to paraquat. Omitoyin *et al*. (2006) stated that gramoxone (paraquat) at a concentration of 18 mg/L caused a median lethal concentration ($LC_{50}$) value after 96 h of exposure. Differences in $LC_{50}$ value between species is due to species-specific toxicity effect and age of the test organisms.

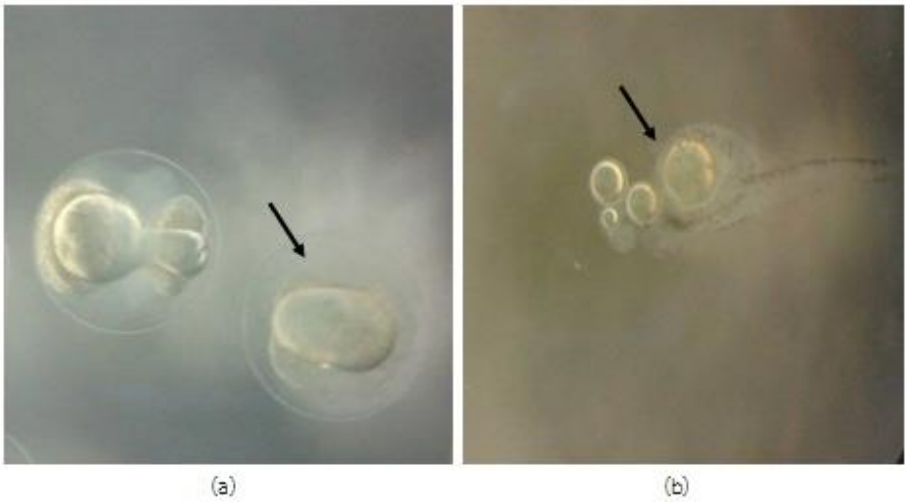


**Fig. 2** Abnormalities in *A. testudineus* embryos. (a) abnormal gastrulation (arrow); (b) yolk sac edema (arrow)

Histological examination of the fingerling stage of *C. carpio* after 96 h of exposure to paraquat at 40 mg/L revealed damage to various organs (Arunlertarce and Kawatsu, 1992). The $LC_{50}$ value was 60 mg/L after 96 h of exposure to the herbicide (Ladipo, 2011). The freshwater fish *Channa punctata* showed significant reduction in the glutathione levels of gills exposed to paraquat at 1.0 mg/L after 24h (Parvez and Raisuddin, 2006). Remarkable differences were observed in paraquat toxicity between pure chemical and formulated commercial products (Arunlertarce and Kawatsu, 1992).The difference in toxicity between pure and formulated commercial products maybe attributable to the other ingredients supplemented in to the formulated products.

The results of our study showed that *A. testudineus* exposed to glyphosate at the highest concentration tested at 0.2 mg/L significantly affected mortality and hatching rate. The silver catfish (*Rhamdia quelen*) exposed to glyphosate at the same tested concentrations showed a significant decrease in brain acetylhydrolase (AChE) activity (Glusczak *et al*., 2007). Dissimilar to our results, Do Carmo Langiano and Martinez (2008) demonstrated that juvenile *Prochilodus lineatus* exposed to Roundup® (glyphosate formulated product) concentrations of 7.5 and 10 mg/L showed sub-lethal results. *A. testudineus* could, therefore, be more sensitive than neotropical fish species. Giesy *et al*. (2000) estimated an acute scenario considering worst-case exposure conditions based on two assumptions: (a) that 2% run off from a 10 Ha field treated at the maximum single use rate of Roundup® cfp5 entered a1 Ha pond 2 m deep, and (b) that 10% of the maximum single application rate per hectare entered the pond through drift, assuming aerial application. Based on these assumptions, maximum concentrations of Roundup® in natural water would range from

0.27 to 0.41 mg/L (Giesy *et al*., 2000). Therefore, high glyphosate concentrations used in this study was environmentally relevant.

Overall, glyphosate showed significantly less lethality than paraquat on the fish embryos with mortality percentage of 38.89 at 12 h of exposure. Similar results were obtained by Deivasugamani (2015) who reported that glyphosate showed less toxic effects on mortality of common carp (*Cyprinus carpio*) than paraquat.

## Conclusions

Results demonstrated that both glyphosate (0.02 to 0.20 mg/L) and paraquat (0.01 to 0.10 mg/L) have a detrimental impact on mortality and hatching rates of *A. testudineus* embryos. Paraquat dichloride was more toxic to *A. testudineus* than glyphosate. Fish can be considered as experimental biological models to measure the environmental impact of different substances. Results indicated that both tested herbicides were detrimental to environmental contamination.

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