

Article History

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Toxicity Test of Turmeric Extract on Wader Fish (*Rasbora* sp.) Embryogenesis Activity

Uji Toksisitas Ekstrak Kunyit terhadap Aktivitas Embriogenesis Ikan Wader (*Rasbora* sp.)

Nur Indah Septriani*, Muhammad Nurul Fathoni Adha, Maftuhatus Sa'diyah, Chelsy Ammara Septiani, Meinawa Amaliah, Tsaqifa Zuhayra Emery Bagus, Soen Eliora Valerie Natania, Enjang Sekaryati Prasetyaningrum

Department of Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia

*Corresponding author: nurindahseptriani@ugm.ac.id

Abstrak

Kunyit (*Curcuma longa*) merupakan tanaman herbal yang mengandung senyawa aktif, terutama kurkumin, yang berpotensi memengaruhi perkembangan embrio ikan, tahap penting bagi keberlangsungan spesies. Sebuah penelitian sebelumnya melaporkan bahwa paparan klorpirifos memengaruhi perkembangan embrio ikan wader pari *Rasbora lateristriata*, sehingga mengonfirmasi kesesuaiannya sebagai model lokal untuk pengujian embriotoksik. Berdasarkan temuan tersebut, penelitian ini mengevaluasi potensi efek toksik ekstrak kunyit pada tahap awal perkembangan embrio. Tingkat toksisitas diuji menggunakan konsentrasi ekstrak kunyit 50 ppm dan 100 ppm pada ikan wader (*Rasbora* sp.). Metode meliputi pembuatan larutan kunyit, pemilihan induk dan pemijahan, pengambilan telur, pembagian kelompok perlakuan, serta pengamatan embrio pada 0, 24, 48, dan 72 jam. Parameter yang diamati meliputi pigmentasi, tingkat mortalitas, dan denyut jantung embrio. Hasil penelitian menunjukkan ekstrak kunyit pada konsentrasi 50 ppm dan 100 ppm memiliki efek toksik terhadap embrio *Rasbora* sp., terutama pada pigmentasi. Semakin tinggi konsentrasi kurkumin, semakin rendah tingkat pigmentasi. Mortalitas embrio mencapai 11,1% pada 50 ppm dan 5,56% pada 100 ppm akibat efek embriotoksik yang mengganggu perkembangan embrio dan menyebabkan koagulasi telur. Denyut jantung sedikit lebih rendah dibandingkan kontrol, menunjukkan pengaruh kurkumin terhadap fungsi jantung dan jalur pensinyalan seluler.

Kata kunci: Denyut jantung; kunyit; mortalitas; pigmentasi

Abstract

Turmeric (Curcuma longa) is a medicinal plant containing active compounds, mainly curcumin, which may affect fish embryonic development, an essential stage for species survival. A previous study reported that exposure to chlorpyrifos affected the embryonic development of the wader pari fish Rasbora lateristriata, confirming its suitability as a local model for embryotoxicity testing. Based on this evidence, the present study evaluated the potential toxic effects of turmeric extract during the early stages of embryo development. Toxicity was assessed using turmeric extract concentrations of 50 ppm and 100 ppm in wader fish (Rasbora sp.). The methods included turmeric solution preparation, broodstock selection and spawning, egg collection, treatment grouping, and embryo observation at 0, 24, 48, and 72 hours. Observed parameters were pigmentation, mortality rate, and embryonic heart rate. The results showed that turmeric extract at both 50 ppm and 100 ppm exhibited toxic effects on Rasbora sp. embryos, especially on pigmentation. Higher curcumin concentrations were associated with lower pigmentation levels. Embryo mortality reached 11.1% at 50 ppm and 5.56% at 100 ppm, caused by embryotoxic effects that disrupted embryonic development and induced egg coagulation. Heart rate was slightly lower than the control group, indicating curcumin's influence on cardiac activity and cellular signaling pathways.

Keywords: heart rate, mortality, pigmentation, turmeric

INTRODUCTION

Embryogenesis is the process by which an embryo is formed and develops. The embryo develops from a single zygote, progressing from a simple unicellular stage to a multicellular organism and eventually reaching the organogenesis stage (formation of body organs). The stages of embryogenesis include cleavage, blastulation, gastrulation, neurulation, and organogenesis [1].

Rasbora sp. (minnow) is a freshwater fish species widely available in nature [2]. This fish belongs to the order *Cypriniformes* and the family *Cyprinidae*. Morphologically, *Rasbora* sp. has a slender, elongated body, a longitudinal stripe extending from the posterior operculum to the base of the tail in yellow or orange with a black underside. Its dorsal scales are golden, while its ventral scales are silvery. Local communities commonly consume this fish due to its pleasant taste and high protein content [3].

In general, female *Rasbora* sp. have larger abdomens than males. This difference is due to females storing numerous eggs for reproduction, resulting in a larger abdominal size compared to males. *Rasbora* sp. spawn twice a year, and seasonal changes influence the timing of spawning. In tropical regions, the rainy and dry seasons affect spawning periods [3].

Curcuma longa (turmeric) is a herbal plant

containing bioactive compounds, primarily curcumin. Curcumin, together with demethoxycurcumin and bisdemethoxycurcumin, belongs to the curcuminoid group. Curcumin exhibits various biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties [4]. Its antioxidant activity protects cells from free radical damage, while its antimicrobial activity acts against various bacteria and fungi commonly found in aquatic environments [5].

Toxicity refers to the ability of a substance to cause damage or death in living organisms [6]. At low concentrations, curcumin and turmeric extract can support fish health by inhibiting the growth of pathogenic microorganisms. However, at specific concentrations, curcumin can exert toxic effects, potentially disrupting physiological functions and cellular development in aquatic organisms [5]. A prior investigation examined the impact of chlorpyrifos on the embryonic development of the wader pari fish *Rasbora lateristriata*, demonstrating that this indigenous species can serve effectively as a model organism for embryotoxicity testing [7]. Building on that evidence, the present study applied turmeric extract to assess its potential adverse effects during the early phases of embryo formation. Specifically, this research aimed to determine the toxicity level of turmeric extract at concentrations of

50 ppm and 100 ppm in *Rasbora* sp.

METHODS

Preparation of Turmeric Solution

The turmeric solution was prepared by weighing 0.1 g of turmeric powder using a precision balance and placing it into a container. Water was heated and then poured over the turmeric powder. The solution was stirred thoroughly to ensure uniform mixing. Subsequently, the solution was diluted to obtain final concentrations of 50 ppm and 100 ppm.

Selection of Broodstock and Spawning

The fish used were 1–2 years old. Twenty *Rasbora* sp. individuals were collected and placed in a basin for sex determination. Sexing was performed using the stripping method by massaging the abdomen. If a white fluid was released from the abdomen, the fish was identified as male; if a yellowish fluid was released, the fish was identified as female. Fish that did not release any fluid were considered not ready for spawning. Fish with determined sex were then placed in a breeding chamber lined with fibers, where they were left overnight to allow natural spawning.

Egg Collection

The morning after spawning, eggs were collected and placed in a basin. Eggs underwent both macroscopic and microscopic screening. Macroscopically, transparent eggs were selected.

Microscopically, healthy eggs at the early cleavage stage were chosen using a light microscope. Selected eggs were then placed in a well plate with media, with 24 eggs per medium. The control group consists of 12 samples, while the treatment group consists of 18 samples, because each wheel unit comprises four rows, and each row contains six wells. In the 5-ppm wheel, one row was allocated for the control and three rows for the treatment, with one embryo placed in each well of the treatment. The same arrangement was applied to the 10-ppm wheel. Therefore, each wheel produced 18 samples for the treatment group, while the total of 12 control samples came from the combined control rows of both the 50 ppm and 100 ppm wheels. The water used in this study was sourced from laboratory tap water, which had a pH of 7.75, a total dissolved solids (TDS) value of 310, and a temperature of 26.6 °C.

Grouping of Fish Embryos

Eggs were distributed into two well plates: the first plate contained turmeric solution at 100 ppm, and the second plate contained turmeric solution at 50 ppm. Each plate included a control group to allow comparison between treated and untreated embryos.

Toxicity Test of Turmeric Solution

The eggs in the well plates were observed over four days using a LEICA microscope at 0, 24, 48, and 72 hours of development. The solution in each well was routinely replaced

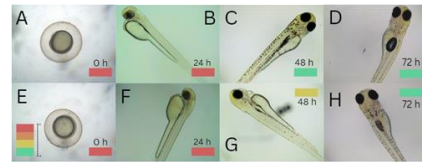
during observations to prevent contamination and maintain solution cleanliness. Observations were documented for further analysis.

RESULTS AND DISCUSSION

Pigmentation

Pigmentation is an essential indicator of larval fish growth and development [8]. In *Rasbora* sp. larvae, the formation of pigments such as melanin and xanthophores is crucial for adapting to varying aquatic conditions, including changes in light intensity and the presence of predators [9]. Pigmentation in *Rasbora* sp. larvae is influenced by environmental factors, including water quality, temperature, and food availability, which can affect pigment development in the larval body [10].

Pigmentation can also be affected by oxidative stress caused by exposure to toxic substances. Toxicity can disrupt normal larval development, including the formation



of

of pigments vital for adaptation and survival. For instance, mercury exposure in fish larvae reduces melanin synthesis, leading to suboptimal pigmentation and decreased resistance to predators [11]. Various toxins can induce the accumulation of free radicals in larval fish, damaging pigment cells and inhibiting the essential synthesis of pigments necessary for survival [12].

In this study, turmeric (*Curcuma longa*) extract, containing the bioactive compound curcumin, was used. Curcumin significantly reduced tyrosinase activity in fish larvae, resulting in decreased melanin production in the skin [13]. This indicates that curcumin can disrupt melanogenesis in fish. The tyrosinase inhibition caused by curcumin may also be associated with increased oxidative stress, which can affect larval health and survival in environments exposed to pollutants.

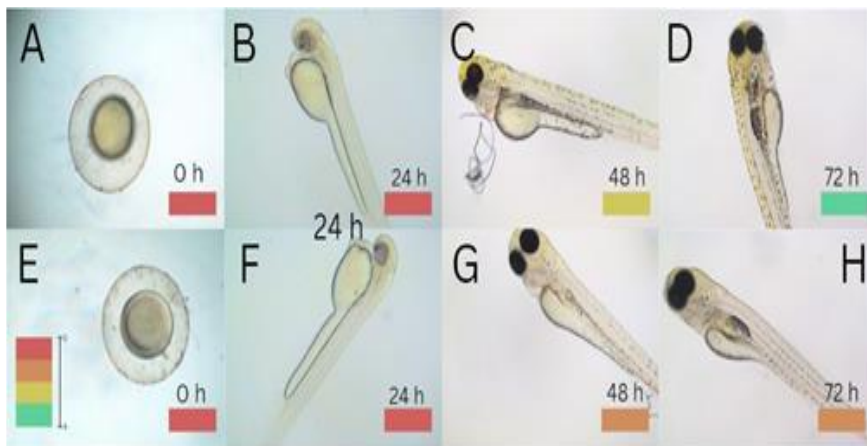


Figure 1. Pigmentation in Control Samples and 50 ppm Turmeric Treatment

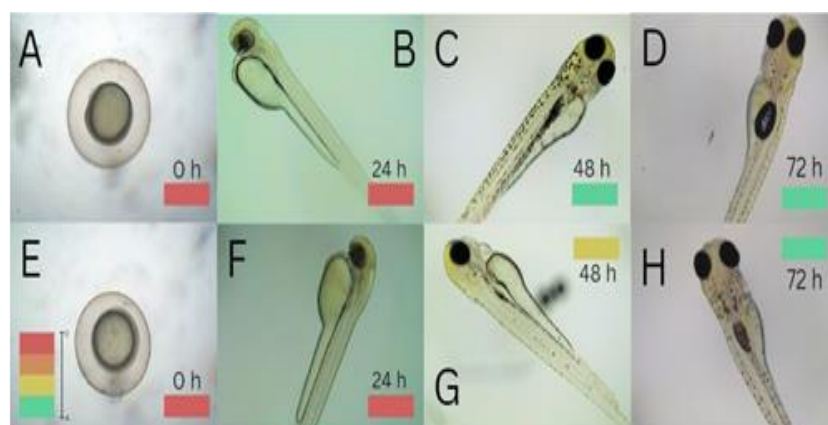


Figure 2. Pigmentation in Control Samples and 100 ppm Turmeric Treatment

Based on the appearance of black spots in *Rasbora sp.*, pigmentation was quantified on a 0–4 scale, where 0 indicates undetectable pigmentation, and 4 indicates high pigmentation. Observations (Figures 1 and 2) showed significant changes in pigmentation at both 50 ppm and 100 ppm turmeric concentrations. At 48–72 hours, the control larvae at 50 ppm exhibited pigmentation at level 1, while treated larvae were at level 2. At 100 ppm, control larvae

had level 4 pigmentation, whereas treated larvae had level 3. The differences correlated linearly with curcumin concentration, suggesting that higher curcumin concentrations reduce pigmentation in *Rasbora sp.*

Mortality

Embryo mortality data for control and treated groups (50 ppm and 100 ppm) are presented in Table 1.

Table 1. Mortality Rate of Wader Fish (*Rasbora sp.*) in Control, 100 ppm, and 50 ppm Turmeric Treatments

Turmeric Treatment	Hour	Initial Number	Mortality Number	Mortality Rate (%)
Control	0	12	0	0
	24	12	0	0
	48	12	0	0
	72	12	0	0
100 ppm	0	18	0	0
	24	18	0	0
	48	18	0	0
	72	18	1	5.56
50 ppm	0	18	0	0
	24	18	2	11.1
	48	18	2	11.1
	72	18	2	11.1

Based on Table 1, in the control group, mortality was 0%, whereas 100 ppm treatment resulted in 5.56% mortality, with one embryo dying at 72 hours. The 50 ppm treatment showed the highest mortality, at 11.1%, between 24 and 72 hours, possibly influenced by abiotic factors such as temperature, pH, and water quality [14].

Live embryos displayed organ formation and movements, while dead embryos appeared opaque white [15]. Coagulation and absence of heartbeats were additional indicators of embryo mortality [16].



Figure 3. Embryo Mortality of Wader Fish under 100 ppm Treatment (A, B) at 14 Hours

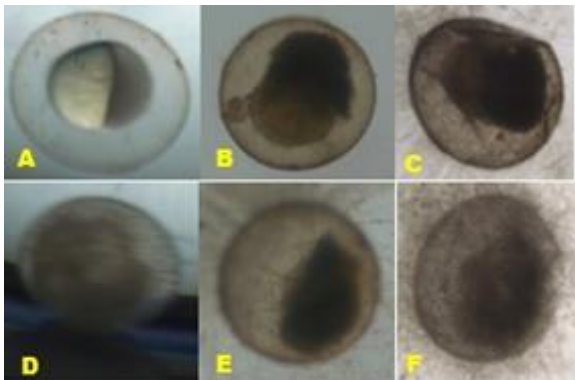


Figure 4. Embryo Mortality of Wader Fish under 50 ppm Treatment (A, B, C) at Treatment 1 and (D, E, F) at Treatment 11

Figures 3 and 4 show embryo mortality at 100 ppm and 50 ppm, respectively, highlighting halted development, inhibited cleavage, and delayed hatching, indicative of early death [17]. These results suggest potential embryotoxic effects of curcumin, possibly linked to oxidative stress and disruption of early cell division [5].

Other Abnormalities

Visual observations revealed that embryos in treatments 1 and 11 exhibited abnormalities. Embryos in treatment one arrested at the blastula stage, with blastomeres appearing rigid and compact, compressing the yolk to form an elliptical shape [18]. Coagulation indicated abnormal eggs, potentially caused by environmental stress, reproductive errors, genetic factors, or handling [19].

Heart Rate

Heart rate measurements of control and treated embryos (50 ppm and 100 ppm turmeric) are presented in Figure 5.

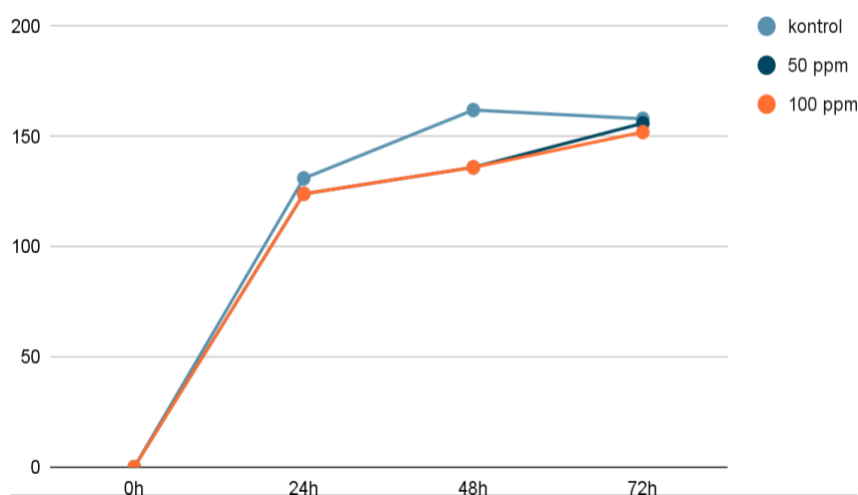


Figure 5. Heartbeat of Wader Fish (*Rasbora* sp.) Embryos at 0–72 Hours of Age

Control embryos exhibited an average heart rate of 131 bpm at 24 hours, increasing to 162 bpm at 48 hours, then slightly decreasing to 158 bpm at 72 hours. Treated embryos (50 ppm and 100 ppm) showed similar trends, with heart rates at 0, 124, 136, and 156 bpm for 50 ppm, and 0, 124, 136, and 152 bpm for 100 ppm at 0, 24, 48, and 72 hours, respectively.

The heart, the first organ to develop in vertebrate embryos, pumps blood throughout the body, supplying oxygen and nutrients. Thus, heart rate is a crucial parameter that reflects the energy-demanding activity of embryonic

development [20]. In *Rasbora* sp., the heart begins beating at ~20 hours post-fertilization, increasing with embryonic age [18]. The slight decrease in heart rate at 50 and 100 ppm turmeric may be due to bioactive compounds in turmeric that modulate blood flow and signaling pathways controlling cell division and activity [21] [22].

Swim Bladder

Based on the observations conducted on wader fish eggs in the control group and those treated with turmeric at 50 and 100 ppm, the results were as follows:

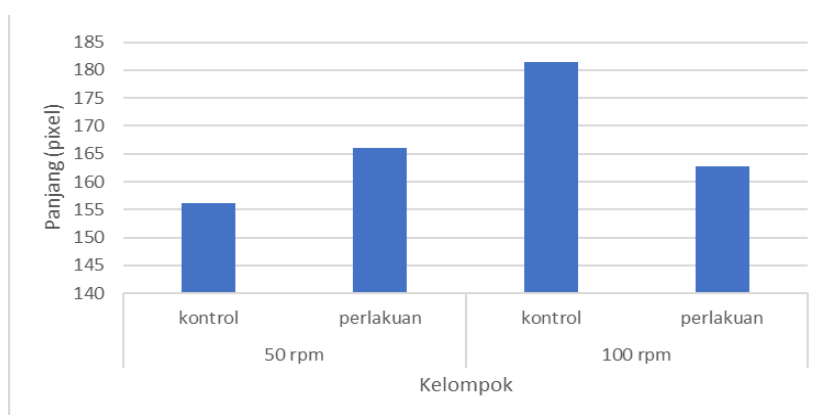


Figure 6. Swim Bladders of Control and Turmeric Treatments (50 & 100 ppm)

The swim bladder is a specialized organ in fish that contains a gas-filled cavity, contributing to buoyancy regulation. It functions as an efficient organ for gas exchange and is also used by fish for respiration. The utilization of the swim bladder as a respiratory organ enables fish to use air as a medium for breathing. Having a swim bladder also reduces the overall body density of fish, thereby lowering the energy expenditure required for swimming [23].

Based on Figure 6, the swim bladder in the 50 ppm treatment group had a higher value than the control group, with 166,069 pixels compared to 156,079 pixels in the control. In contrast, the 100 ppm treatment group had a lower value than the control, with 162,796 pixels compared to 181,472 pixels in the control.

CONCLUSION

Based on the results and discussion, it can be concluded that turmeric (*Curcuma longa*) at concentrations of 50 ppm and 100 ppm exerts toxic effects on *Rasbora sp.*, particularly on pigmentation, embryo mortality, and heart rate. Higher concentrations of curcumin led to reduced pigmentation levels in *Rasbora sp.* larvae. Embryo mortality was associated with the embryotoxic effects of curcumin, which disrupted embryonic development and induced egg coagulation. Heart rate measurements at 50 ppm and 100 ppm were

slightly lower than in the control group, indicating that curcumin influenced cardiac activity and signaling pathways involved in cellular activity and division. Additionally, the swim bladder was observed to be larger at 50 ppm compared to 100 ppm.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this scientific work.

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