



Effect of Ketapang (*Terminalia catappa*) Leaf Extract Soaking on the Hatching Rate and Survival Rate of Fertilized Gouramy Fish Eggs (*Osphronemus goramy*)

Wildan Rasyid Mukhtar^{1*}, Sri Hastuti¹, Dewi Nurhayati¹

¹Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Indonesia

*Corresponding author: wildanrasyid212@gmail.com

Received 20 December 2023; Revised 4 February 2026, Accepted 26 February 2026;
Available online 10 March 2026, Published 10 March 2026

ABSTRACT

Low egg hatching rates caused by fungal infections are a common problem in the incubation of gourami eggs. One method to overcome this problem is soaking the eggs in ketapang (*Terminalia catappa*) leaf extract, which has antifungal compounds. This study aimed to determine the effect of soaking gourami eggs (*Osphronemus goramy*) in ketapang leaf extract at different doses on hatching rate. The experiment was conducted using a completely randomized design (CRD) with four treatments and three replications. Treatment A, B, C, and D used ketapang leaf extract at concentrations of 0, 1, 2, and 3 g/L, respectively. [L1.1]The observed parameters included egg hatchability, larval survival rate, fungal prevalence on eggs, and water quality parameters of the hatching and larval rearing media (temperature and pH). The results showed that soaking eggs in ketapang leaf extract had a significant effect ($P < 0.05$) on egg hatchability, fungal prevalence, and larval survival rate. Treatment C at a dose of 2 g/L was the most effective, producing the highest hatchability ($88.33 \pm 2.08\%$), the lowest fungal prevalence ($10.00 \pm 3.51\%$), and the highest larval survival rate ($81.20 \pm 0.74\%$). Water quality parameters were within suitable ranges, with temperatures of 23–28 °C and pH values of 6.7–7.5.

Keywords: Gouramy, ketapang leaf extract, hatching rate, fungus prevalence, survival rate

ABSTRAK

Permasalahan yang sering terjadi pada proses penetasan telur ikan gurame yaitu daya tetas telur yang rendah akibat serangan jamur. Salah satu cara untuk mengatasi hal tersebut adalah dengan melakukan perendaman telur dengan ekstrak daun ketapang sebagai bahan yang memberikan efek antijamur. Penelitian ini bertujuan untuk mengetahui pengaruh perendaman telur ikan gurame (*Osphronemus goramy*) dalam ekstrak daun ketapang (*Terminalia catappa*) dengan dosis berbeda terhadap daya tetas. Rancangan penelitian yang digunakan yaitu rancangan acak lengkap (RAL), dengan 4 perlakuan dan 3 ulangan. Perlakuan A menggunakan ekstrak daun ketapang 0 g/L, perlakuan B 1 g/L, perlakuan C 2 g/L, perlakuan D 3 g/L. Jumlah telur yang digunakan yaitu 100 butir telur per liter. Parameter yang diamati pada penelitian ini yaitu: daya tetas telur, kelulushidupan larva, prevalensi jamur pada telur dan parameter kualitas air media penetasan telur dan pemeliharaan larva (suhu dan pH). Hasil penelitian menunjukkan bahwa perendaman telur dengan ekstrak daun ketapang berpengaruh nyata ($P < 0,05$) terhadap daya tetas telur, prevalensi jamur dan *survival rate*. Perlakuan C dengan dosis 2 g/L merupakan dosis terbaik yang menghasilkan nilai daya tetas tertinggi yaitu $88,33 \pm 2,08\%$, prevalensi infeksi jamur terendah yaitu $10,00 \pm 3,51\%$, dan kelangsungan hidup larva tertinggi yaitu $81,20 \pm 0,74\%$. Kualitas air untuk suhu berkisar antara 23-28 °C dan pH berkisar antara 6,7-7,5.

Kata kunci: Ikan gurame, ekstrak daun ketapang, daya tetas, prevalensi jamur, kelulushidupan

1. Introduction

Gourami (*Osphronemus goramy*) is an endogenous freshwater fish that is widely cultivated in Indonesia. Gourami fish have become a cultivated commodity that plays a role in the economy and improving community nutrition. Although gourami is one of the main freshwater aquaculture commodities in Indonesia, its production remains lower than that of tilapia, catfish, common carp, and pangasius; however, an increasing trend has been observed. Gourami production reached approximately 149,169 tons in 2021 and increased to 156,309 tons in 2024 (MMAF, 2025). Gourami prices remain relatively stable due to sustained high demand. In 2026, e-commerce data indicated that retail prices in Indonesia ranged from 45,000 IDR to 65,000 IDR, depending on the seller, location, and freshness of the product. The demand for consumption of gourami fish for the market in Jakarta reaches 10–15 tons per day. According to Simanjuntak et al. (2021), although the price of gourami fish is relatively higher compared to other freshwater fish, gourami fish still have their charm and are still in demand by the public, so gourami cultivation has high prospects.

An important role in gourami cultivation lies in the hatching process. One of the obstacles often faced in the seeding process is the low hatchability of eggs caused by fungal attacks. According to SNI 1-6485.3.2000, the hatchability of gourami fish eggs is around 80–95%. However, in reality, the hatchability of gourami fish is still far below this figure, reaching 60% (Ghofur et al., 2014). One of the major challenges in gourami aquaculture is ensuring a consistent supply of high-quality seed for fish farmers. In gourami hatcheries, one of the key factors affecting seed availability is the hatching success of gourami eggs. One of the infectious diseases that negatively affects egg hatching success is fungal infection. Cotton wool disease, characterized by white cotton-like growths that adhere to the affected areas, is the most common fungal infection (Sarkar et al., 2022). *Saprolegnia* species are the most frequently encountered fungi and are capable of infecting fish eggs by swimming from dead eggs to live eggs through positive chemotaxis (Sarkar et al., 2022). Fungi can attach to and penetrate the chorion, weaken the eggs, and absorb essential nutrients such as glycoproteins and lipoproteins, through virulence factors such as subtilisin-like serine protease (SpSsp1), cellulose-binding domain, GH F17, and endo-1,3- β -glucanase (gene *PIEndo1*) (Torto-Alalibo et al., 2005). Therefore, the application of preventive methods during egg development is crucial to inhibit fungal infection.

Currently, preventive measures against fungal infections include soaking fish eggs in antifungal agents. The soaking process often

involves the use of synthetic compounds, such as methylene blue, malachite green, and povidone-iodine (Ghofur et al., 2014). One of the methods commonly used to control fungal infections on freshwater fish eggs is malachite green, formalin, acetic acid methylene blue, and hydrogen peroxide (Koo, 2000; Ma et al., 2020). The use of these chemicals may negatively affect egg development and fish at subsequent life stages, and may also lead to environmental contamination (Lahnsteiner and Dünser, 2025). In aquaculture, malachite green has been widely used in the freshwater fish farming industry to prevent fungal infections (Srivastava et al., 2004). However, malachite green is not recommended due to its genotoxic and carcinogenic properties (Rao, 1995), and its large-scale use has the potential to cause harm and pollute aquatic ecosystems (Majeed et al., 2014). The use of this compound has been banned by the European Union (Niska et al., 2009) as well as by the United States Food and Drug Administration (US Food and Drug Administration) (Srivastava et al., 2004). Therefore, it is important to explore safer alternative antifungal antimicrobial agents.

The continuous use of synthetic materials can cause problems for the environment, so there is a need for alternative materials that are effective in dealing with fungal attacks but are still environmentally friendly. One alternative ingredient that can prevent fungal attacks is ketapang leaves (*Terminalia catappa*). This is because ketapang leaves are easy to obtain and are commonly used to treat diseases in fish. Ramadhian et al. (2017) stated that ketapang leaves contain compounds such as phyosterols, flavonoids, saponins, and tannins, which function as anti-fungal agents. The application of natural ingredients in the field of fish diseases, especially gourami fish, has been proven to be able to replace synthetic materials to overcome disease attacks. According to research conducted by Triwardani et al. (2022), soaking tawes fish eggs in ketapang leaf solution had a significant effect on the hatchability and survival of larvae. In research conducted by Saenal et al. (2020), soaking goldfish eggs in ketapang leaf solution can increase hatchability due to the antimicrobial content in the ketapang leaf solution, which protects goldfish eggs. Based on these considerations, this study investigated the application of ketapang (*Terminalia catappa*) leaf extract as a soaking treatment for gourami fish eggs to evaluate its effects on egg hatchability and larval survival.

2. Materials and methods

2.1 Fish eggs

The materials used in the research were gourami fish eggs (*O. goramy*) of the hybrid strain obtained from gourami fish farmers

around the research location with a total number of 1,200 eggs [L2.1] with a stocking density of 100 eggs per liter, water originating from local water sources. The research was conducted at the Integrated Laboratory, Diponegoro University, Indonesia. [L3.1].

Gourami eggs were collected from the nest (sosog) and subsequently transferred to a separate container. The eggs were transferred carefully to avoid damage. A total of 1,200 fertilized gourami eggs were used and allocated to 12 treatment containers at a density of 100 eggs L⁻¹ per treatment. The containers used in the research were 9 1-liter beaker glass for soaking gourami fish eggs. Then 12 buckets with a diameter of 60 cm as maintenance containers, aerators and equipment to supply oxygen, blenders for grinding ketapang leaves, digital scales, filter cloth, glass bottles, erlenmeyer flask, rotary evaporator, thermometer to measure temperature, and pH meter to measure pH, and digital camera as a documentation tool.

2.2 Ketapang leaf extract

The extraction process of ketapang leaf (*T. catappa*) was conducted at the Integrated Laboratory of Diponegoro University. The method of extraction followed the method described by Herli and Wardaniati (2019), using the maceration technique with ethanol as the solvent. Ketapang leaves were cleaned and sun-dried, after which the dried leaves were ground into powder (simplicia). The simplicia were soaked in ethanol for 24 hours at a ratio of 1:2 (w/v). The extract was then filtered using filter cloth and collected in an Erlenmeyer flask. The filtrate was concentrated using a rotary evaporator to remove the ethanol solvent. The resulting ketapang leaf extract was subsequently subjected to phytochemical analysis to determine its tannin content. Qualitative phytochemical testing was performed by adding FeCl₃ reagent; the formation of a blackish-green color indicated a positive result for tannins (Nofitarini et al., 2019).

2.3 Experimental Design

The method used in this research was a completely randomised design with 4 treatments and 3 replications. The dose treatment of ketapang leaf extract above refers to research conducted by Saenal et al. (2020) which states that soaking goldfish eggs (*Cyprinus carpio*) with a solution of Ketapang leaves (*T. catappa*) has a significant effect on the hatchability of goldfish eggs with a dose of

solution in soaking which is as much as 2 g/L. The treatment given was the addition of ketapang leaf extract to the soaking medium, with concentrations, A (0 g/L), B (1 g/L), C (2 g/L), and D (3 g/L).

2.4 Soaking and hatching of eggs

The egg soaking process began with the preparation of labeled glass beakers as soaking containers for each treatment. Each container was filled with 1 L of water and supplemented with ketapang leaf extract at the designated dose for each treatment, measured using a digital scale. The eggs were then transferred to a soaking container for each treatment. The length of soaking time refers to research by Saenal et al. (2020), which is carried out for 25 minutes. After the soaking process, the eggs are then moved to a hatching container filled with clean water, given aeration and then left to sit until the eggs hatch. The larvae that have hatched are then kept for 15 days to determine the survival rate.

2.5 Observed parameters

The research variables observed included tannin phytochemical tests, hatching rate (HR), fungal prevalence, survival rate (SR) and water quality. The phytochemical test was carried out at the Integrated Laboratory of Diponegoro University qualitatively by adding FeCl₃ reagent, if a blackish-green color is formed, it shows a positive tannin result (Nofitarini et al., 2019).

Egg hatchability is calculated using the formula proposed by Rustidja (1997), which is as follows:

$$HR (\%) = \frac{\text{hatched eggs}}{\text{fertilized eggs}} \times 100\%$$

Observations on the prevalence of fungus were carried out on eggs affected by fungus, with the characteristics of white hyphae attached to the eggs. According to Andreas (2016), prevalence is calculated using the following formula:

$$\text{Prevalence} = \frac{N \times 100\%}{n}$$

Information:

N: Number of samples infected with fungus

n: Number of samples observed

The larval survival rate is calculated using the formula proposed by Effendi (1997) which is carried out when the larvae reach 15 days of age, namely as follows:

$$SR (\%) = \frac{\text{Larvae at the end}}{\text{Larvae at the start}} \times 100\%$$

During the research, water quality was measured in several parameters, including temperature and acidity (pH). Water quality is measured using a thermometer and pH meter. Water quality measurements were carried out in the morning and evening in each container for hatching eggs and rearing gourami larvae.

2.6 Data Analysis

The results obtained during the study: hatching rate (HR), survival rate (SR), fungal prevalence and water quality. The data obtained from the results of the study were analysed statistically by conducting normality test, homogeneity test, additivity test, and analysis of variance (ANOVA) using SPSS 25 software. ANOVA analysis test was conducted on the data of hatching rate and survival rate to determine the effect of leaf extract on egg hatchability. If the results of the analysis showed a significantly different effect, the test was continued with the Duncan test at the 5% level to determine the difference in the mean value between treatments. While water quality was tested descriptively.

3. Results and Discussion

3.1. Phytochemical Test

Based on the results of phytochemical test research on ketapang leaf extract (*T. catappa*) that was carried out, we got positive results (+) that the extract contained tannin. The phytochemical test carried out on Ketapang leaf extract was carried out to determine the content of tannin compounds contained in ketapang leaves [L6.1]. The presence of tannin content in ketapang leaf extract [L7.1] can be indicated if a blackish-green color forms after adding FeCl_3 reagent (Nofitarini et al., 2019).

Plant leaves generally contain terpenes, flavonoids, tannins, alkaloids, steroids, proteins, carbohydrates, fats, and oils (Hassan et al., 2020). The tannin compounds contained in ketapang leaf extract play an important role in inhibiting fungal growth on gourami fish eggs. This finding is supported by Diansyah and Diana (2017), who reported that tannin compounds can dissolve in lipids and function as anti-fungal compounds. Tannins function as antimicrobial compounds. Ozogul et al. (2025) explained that tannins affect physiological functions related to membrane transport, particularly osmotolerance and osmoprotection. In addition, tannins increase membrane permeability and induce acidification of cellular components through the downregulation of genes responsible for pH homeostasis,

especially those involved in catalyzing Na^+ efflux and proton uptake. This condition reduces nutrient uptake (amino acid absorption) from the environment, leading to disruptions in energy metabolism as well as impaired iron uptake, recognition, and sequestration through gene regulation, ultimately resulting in iron deficiency that contributes to microbial cell lysis (Ozogul et al., 2025).

3.2. Hatching Rate

The hatching rate (HR) of gourami (*O. goramy*) eggs is presented in **Figure 1**. Based on the results of research that has been carried out, showed that soaking eggs in ketapang (*T. catappa*) leaf extract at different doses can have a significant effect ($P < 0.05$) on the hatchability of gourami eggs. It can be seen in **Figure 1** above that immersion in treatment C with a concentration of 2 g/L produces the highest hatching rate value, namely $88.33 \pm 2.08\%$. Then continued with treatment D (3 g/L), B (1 g/L) and A (0 g/L) with values respectively $84.67 \pm 4.51\%$, $78.67 \pm 5.13\%$ and $73.67 \pm 3.51\%$. These results are in accordance with research by Triwardani et al. (2022) which states that treatment with a dose of 2.0 ml/l is the best treatment by producing the highest hatchability value.

Treatment A showed a small hatchability value due to the lack of protection for the eggs against fungi. Fungi infect eggs through spores that stick to and enter the egg through virulence factors such as subtilisin-like serine protease (SpSsp1), cellulose-binding domain, GH F17, and endo-1,3- β -glucanase (gene *PiEndo1*) (Torto-Alalibo et al., 2005). The spores that enter then reproduce by absorbing nutrients in the eggs. Eggs that have been infected with fungus will appear to have a white net surrounding the egg. Susilo and Yusanti (2022) added that if fungal growth is not controlled, it can spread to infect other healthy eggs and cause a high egg death rate. Then in treatment B, the amount of ketapang leaf extract was still too small so the anti-fungal protection in the ketapang leaf extract was not evenly distributed and caused the hatchability value to be also small. In treatment C, gourami fish eggs appeared to be better protected by the anti-fungal substances in ketapang leaf extract, resulting in high hatchability values. Tannin is a compound that can help protect eggs from fungal infections. The effect of soaking Ketapang leaf extract on the hatchability of gourami eggs is due to the tannin content in Ketapang leaf extract. This is in accordance-

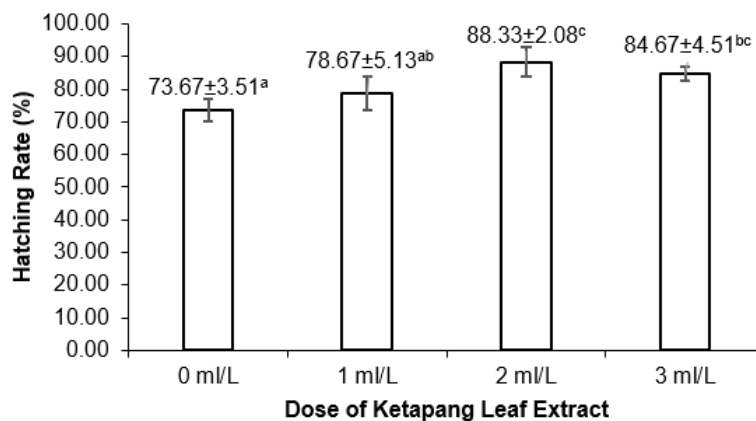


Figure 1. Hatching rate (HR) of Gourami eggs (*O. Gouramy*) after soaking with Ketapang leaf extract. Means with different superscripts indicate significantly different ($P < 0.05$)

with the statement of Saenal *et al.* (2020), that the tannin content in ketapang leaves can protect from fungal attacks during soaking which can cause the eggs to die.

Meanwhile, treatment D obtained a lower hatchability value than treatment C, which was thought to be due to the concentration of the extract being too high, causing embryo development to be too fast, making the embryos premature. Because besides tannins, ketapang leaves are known to contain several compounds such as flavonoids, saponins, and phenolics. Flavonoids are a class of naturally occurring phenolic compounds derived from plants (phytochemicals) that originate from flavone and possess antioxidant properties (Nwosu *et al.*, 2022).

These compounds are widely found in various plants, fruits, herbs, stems, cereals, nuts, vegetables, flowers, seeds, and leaves, and have potential applications in medicinal chemistry (Edo, 2022). The mechanisms by which flavonoids exert anti-inflammatory and antioxidant effects include scavenging or neutralizing free radicals, chelating metal ions, and inhibiting enzyme systems responsible for the generation of free radicals (Pizzino *et al.*, 2017).

Beside that, saponin compound can degrade the membrane or cell wall of the egg so that the larvae in the egg are released and hatching time of the fish becomes faster. Inaya *et al.*, (2015) stated that saponins can reduce the egg membrane so that there is a change in the structure of the egg cell wall which results in the liquid in the cell coming out. This is in accordance with the statement of Ghofur *et al.* (2014) which states that soaking with too much extract concentration will make the embryos unable to adapt for longer and die during the hatching phase.

3.3. Fungal Prevalence

The results of observing the prevalence of fungi in gourami (*O. goramy*) eggs are presented in **Figure 2**. The infection developed naturally, and no experimental challenge test was conducted. Based on the results of research obtained, [L10.1] the highest fungal prevalence value was found [L11.1] in treatment A (0 g/L), namely $24.67 \pm 4.73\%$, followed by treatment B (1 g/L) at $15.00 \pm 1.00\%$, treatment D (3 g/L) [L12.1] was $10.67 \pm 3.61\%$, and treatment C (2 g/L) was $10.00 \pm 3.51\%$.

In treatment A, the eggs were not protected at all by anti-fungal compounds, resulting in the highest fungal prevalence values. This is in accordance with research by Yuniar *et al.* (2022) which states that treatment of eggs with anti-fungal compounds causes the eggs to be better protected from fungal attacks compared to treatment without anti-fungal compounds. The use of extract of *T. catappa* enhanced its effectiveness in protecting eggs and preventing fungal proliferation due to its chemical profile containing polyphenols (flavonoids) and tannins (gallic acid, ellagic acid, α - and β -punicalagin), which exhibit fungicidal effects (Meneses *et al.*, 2021). Flavonoids form complex compounds with proteins that can penetrate and lyse the cell membrane, thereby inhibiting fungal growth (Sulistyawati and Sri, 2009).

The evaluation of fungal prevalence was conducted based on visual examination of gourami eggs. Eggs that appeared whitish and exhibited the growth of white, cotton-like hyphae were considered to be infected with fungi. Microscopic identification of the infecting fungal species was not conducted in this study. Oomycetes such as *Saprolegnia* spp. and *Aphanomyces* spp. are important aquatic pathogens that can cause diseases in fish eggs, with *Saprolegnia* being the primary causative agent of saprolegniasis in fish eggs (Koo,

2000). Other studies have shown that *Saprolegnia* can infect fish eggs of brown trout (*Salmo trutta*) (Rezinciuc et al., 2014), white fish (*Rutilus frisii kutum*) (Kalatehjari et al., 2015), and angelfish (*Pterophyllum scalare*) (Meneses et al., 2021). Saenal et al. (2020) stated that the tannin content in ketapang leaves has antimicrobial properties that prevent egg death due to attack by the fungus *Saprolegnia sp.*

3.4. Survival Rate

The results of observations of survival (SR) of gourami fish larvae (*O. goramy*) are presented in Figure 3. Based on the results of research that has been carried out, treatments C (2 g/L) and D (3 g/L) produce the highest survival rate values, namely $81.20 \pm 0.74\%$ and $79.13 \pm 1.93\%$, respectively—then continued with treatment B (1 g/L) with a value of $75.26 \pm 5.81\%$ and treatment A (0 g/L) with a value of 50.33% . These results indicated that immersion of eggs in ketapang leaf extract at doses ranging from 1 to 3 g/L is non-toxic, did not adversely affect embryonic development, and had a positive effect on the survival rate of gourami larvae. This is in accordance with research by Triwardani et al. (2022) who stated that soaking tawes fish eggs in ketapang leaf solution resulted in the highest survival rate in treatment with a dose of 2 ml/l. The influence of Ketapang leaf extract on the survival rate is thought to be due to good protection by the tannin content in Ketapang leaf extract so that the eggs can be protected from fungus. This is confirmed by Bowo et al. (2014) who stated that ketepang leaves which contain tannin can act as an anti-fungal so that the eggs become healthy and the survival of larvae increased.

SNI: 1-6485.3.2000 stated that gourami larvae's suitable survival value is between 70 and 80%. Therefore, treatment C's survival rate of 70.67% passes the criteria. The percentage of carp larvae that survive can also be influenced by the hatchability of the eggs. A low proportion of hatchability will also result in a low rate of larval survival (Triwardani et al., 2022).

The application of ketapang leaf extract to the egg has no adverse effects on the gourami larvae, as indicated by the high larval survival value, which shows that the larval development in the egg is growing properly. The claim made by Triwardani et al. (2022) that providing eggs with ketapang leaf solution at a suitable dosage has no negative impact confirms this.

3.5. Water Quality

Water temperature of the rearing tank [L14.1] is one of the main environmental factors influencing fish physiology from the pre-hatching stage (Teletchea et al., 2009). Observation data on water quality in the hatching and rearing containers for gourami fish (*O. goramy*) larvae are presented in **Table 1**. Based on the results of measurements carried out during the research, the temperature ranged from 23-28°C [L15.1]. This was considered [L16.1] unfavorable because the minimum temperature recorded is less than 25°C. According to Jumaidi et al. (2016), gourami fish will live well if the water temperature is around 25-30°C. The low temperature recorded during these observations is still said to be not too dangerous for gourami larvae because the water temperature is not too cold. In fish hatcheries, exceeding the optimal temperature tolerance range can lead to negative effects, including physiological disturbances and increased susceptibility to diseases, and may potentially result in mortality (Singh et al., 2013).

Based on the measurement results, the pH value obtained was recorded at around 6.7-7.5. This is still considered quite normal and safe for hatching eggs and rearing gourami larvae. A pH value that is too high or too low will disrupt fish growth and can even cause death. According to Irawan et al. (2019), an inappropriate pH value can affect water conditions which will change the nature of the water to become toxic and result in fish experiencing slow growth and being sensitive to bacteria and parasites.

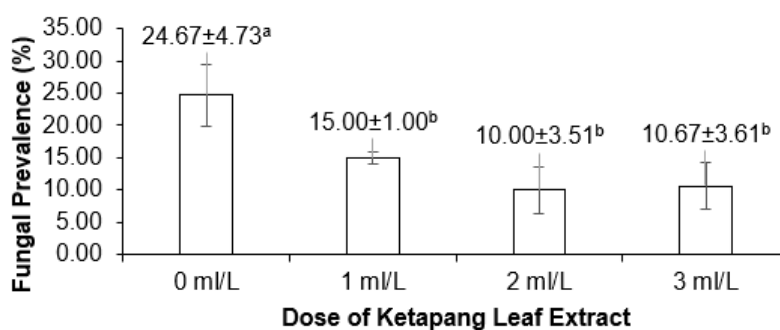


Figure 2. Histogram of fungal prevalence of Gourami eggs (*O. Gouramy*) after soaking with Ketapang leaf extract. Means with different superscripts indicate significantly different ($P < 0.05$).

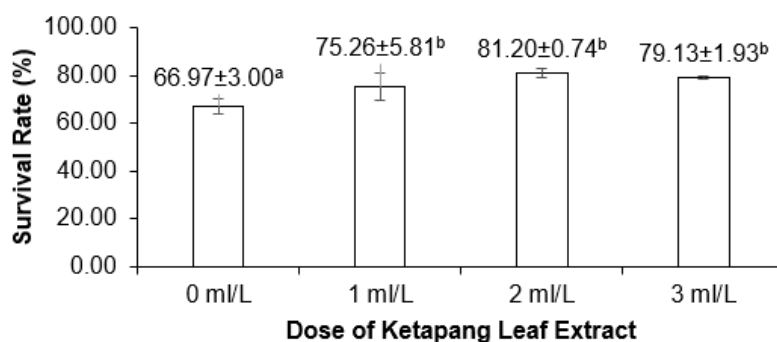


Figure 3. Survival rate (SR) of Gouramy Eggs (*O. Gouramy*) after soaking with Ketapang leaf extract. Means with different superscripts indicate significantly different ($P < 0.05$).

Table 1. Water quality parameters during experiment

Treatment	Range of water quality parameter value	
	Temperature (°C)	pH
0 g/L (A)	24-26	7,0-7,3
1 g/L (B)	23-28	6,8-7,5
2 g/L (C)	24-28	6,8-7,4
3 g/L (D)	25-27	6,7-7,4
Qualification Value	25-30 ^a	6,5-8,0 ^a

Note: ^a: SNI: 01-7241-2006

4. Conclusion

Based on the research conducted, it has been determined that soaking gourami fish eggs in ketapang leaf extract can actually affect the hatchability of the eggs, the amount of fungi present, and the survival of the gourami fish larvae (*O. gouramy*). The recommended dose for producing the highest hatchability value ($88.33 \pm 2.08\%$), lowest prevalence of herbal medicine ($10.00 \pm 3.51\%$), and highest larval survival rate ($81.20 \pm 0.74\%$) was 2 g/L in treatment C.

Acknowledgements

The author thanks Dr. Ir. Desrina, M.Sc. and Dr. Diana Chilmawati, S.Pi., M.Si. for all the critics and suggestions, Integrated Laboratory of Diponegoro University, the Head and all Staff of the UPT Balai Pengembangan Budidaya Air Tawar (BPBAT) Dinas Perikanan dan Peternakan, Banyumas Regency and all parties who have helped in this research which cannot be mentioned one by one.

References

Andreas, M.S. 2016. Identification and prevalence of fungi in gourami

(*Osphronemus gouramy*) in modern markets of Surabaya. [Undergraduate Thesis]. Faculty of Fisheries and Marine Sciences, Airlangga University, Surabaya, 45 pp.

- Bowo, A.T., Sunarto, and Rachimi. 2014. Effect of ketapang leaf extract (*Cassia alata* L.) on the prevention of *Saprolegnia sp.* fungal infection in gourami (*Osphronemus gouramy*) eggs. *Jurnal Ruaya*, 1(1): 8–16.
- Diansyah, S. and F. Diana. 2017. Effect of *Tagetes erecta* L. extract on the growth of *Saprolegnia sp.* fungus in vitro. *Jurnal Perikanan Tropis*, 4(2): 199–208.
- Edo, I.G. 2022. Analysis of phytochemical constituents and antioxidant potential of bitter kola leaf extract towards bioactive food, nutrition and health resources. *Organic & Medicinal Chemistry International Journal*, 11(5). <https://doi.org/10.19080/OMCIJ.2022.11.5.55823>.
- Effendi, M. 1997. *Fisheries biology*. Yayasan Pustaka Nusantara, Yogyakarta, 163 pp.
- Ghofur, M., M. Sugihartono, and R. Thomas. 2014. Effectiveness of betel leaf extract (*Piper betle* L.) on the hatching of gourami (*Osphronemus gouramy*) eggs. *Jurnal Ilmiah Universitas Batanghari Jambi*, 14(1): 37–44.
- Hadi, S., M. Junaidi, and D.P. Lestari. 2022. Embryogenesis stages of gourami (*Osphronemus gouramy*) eggs at different temperatures for 48 hours. *Jurnal Media Akuakultur*, 2(2): 177–189.
- Inaya, A., F.N. Kismiyati, and S. Subekti. 2015. Effect of papaya seed extract (*Carica papaya*) on egg damage of *Argulus japonicus*. *Jurnal Ilmiah Perikanan dan Kelautan*, 7(2): 159–164.
- Hassan, A., Akmal, Z., & Khan, N. 2020. The phytochemical screening and antioxidants potential of *Schoenoplectus triqueter* L.

- Palla. *Journal of Chemistry*, 2020: 1–8. <https://doi.org/10.1155/2020/3865139>.
- Hossen, M.S., A.H.M.M. Reza, S.F. Rakhi, M.M. Rahman, M.A. Alam, and Z. Hossain. 2014. Observation of embryonic and early larval development of striped gourami, *Trichogaster fasciata* (Perciformes: Osphronemidae). *EurAsian Journal of BioSciences*, 8: 61–70.
- Irawan, D., S.P. Sari, E. Prasetyono, and A.F. Syarif. Growth performance and survival of Seluang fish (*Rasbora einthovenii*) under different pH treatments. *Journal of Aquatropica Asia*, 4(2): 15–21.
- Jumaidi, A., H. Yulianto, and E. Efendi. 2016. Effect of water flow rate on water quality improvement in a recirculation system and its relationship with survival and growth of gourami (*Osphronemus gouramy*) fingerlings. *Jurnal Rekayasa dan Teknologi Budidaya Perairan*, 5(1): 587–596.
- Kalatehjari, P., Yousefian, M., and Khalilzadeh, M.A. 2015. Assessment of antifungal effects of copper nanoparticles on the growth of the fungus *Saprolegnia sp.* on white fish (*Rutilus frisii kutum*) eggs. *Egyptian Journal of Aquatic Research*, 41: 303–306.
- Khoo, L. 2000. Fungal diseases in fish. *Seminars in Avian and Exotic Pet Medicine*, 9(2): 102–111.
- Lahnsteiner, F., Dünser, A. 2025. A simplified one-step technique for disinfection of non-hardened rainbow trout eggs with tosylchloramide (Chloramine T) and peroxide (Wofasteril) compounds and the effects on bacterial load and microbiome composition compared to iodophore disinfection. *Journal of Genetic Engineering and Biotechnology*, 23: 100541.
- Ma, R., Fang, W., Zhang, H., Sun, J., Su, H., Chen, T., and Hu, K. 2020. Transcriptome analysis of zebrafish (*Danio rerio*) eggs following treatment with malachite green. *Aquaculture*, 514: 734500.
- Majeed, S.A., Nambi, K., Taju, G., Vimal, S., Venkatesan, C., Hameed, A.S.S., 2014. Cytotoxicity, genotoxicity, and oxidative stress of malachite green on the kidney and gill cell lines of freshwater air-breathing fish *Channa striata*. *Environmental Science and Pollution Research* 21, 13539–13550.
- Meneses, J.O., da Silva, I.C.A., da Cunha, A.V.S., de Souza, J.C.N., Nascimento, V.R.S., Santos, C.C.M., Cunha, F.D.S., Paixão, P.E.G., Dias, J.A.R., Sousa, N.D.C., do Couto, M.V.S., Abe, H.A., Filho, R.M.N., Santos, T.B.R., Santos, F.J.D., da Costa, L.P., Cardoso, J.C., Fujimoto, R.Y., 2021. Efficacy of *Terminalia catappa*-AgNP nanocomposite against *Saprolegnia parasitica* infection in angelfish (*Pterophyllum scalare*) eggs. *Aquaculture* 543, 736914.
- Ministry of Marine Affairs and Fisheries (MMAF), 2025. Fish production statistics portal. <https://portaldata.kkp.go.id/portals/data-statistik/prod-ikan/tbl-statis/d/53> (accessed 31 January 2026).
- Niska, K., Korkea-Aho, T., Lindfors, E., Kiuru, T., Tuomainen, M., Taskinen, J., Peltonen, K., 2009. Disappearance of malachite green residues in fry of rainbow trout (*Oncorhynchus mykiss*) after treatment of eggs at the hatching stage. *Aquaculture* 297, 25–30.
- Nofitarani, R., Novita, F.S., Hidayah, F.N., 2019. Qualitative test of alkaloids and tannins in onion peel and ketapang leaf extracts using the ultrasonic extraction method. *Proceedings of the 10th SNST*, 35–39.
- Mustofa, A.R., Nugroho, R.A., Subandiyono, 2022. Effect of flavonoids and curcumin in turmeric extract as a soaking medium on the hatching rate of gourami (*Osphronemus gouramy*) eggs. *Jurnal Sains Akuakultur Tropis* 6(2), 246–254.
- Nwosu, L.C., Edo, G.I., Özgör, E., 2022. The phytochemical, proximate, pharmacological, and GC–MS analysis of *Cyperus esculentus* (tiger nut): A fully validated approach in health, food, and nutrition. *Food Bioscience* 46, 101551. <https://doi.org/10.1016/j.fbio.2022.101551>
- Ozogul, Y., Ucar, Y., Tadesse, E.E., Rathod, N., Kulawik, N., Trif, M., Esatbeyoglu, T., Ozogul, F., 2025. Tannins for food preservation and human health: A review of current knowledge. *Applied Food Research* 5, 100738.
- Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., et al., 2017. Oxidative stress: Harms and benefits for human health. *Oxidative Medicine and Cellular Longevity* 2017, 1–13. <https://doi.org/10.1155/2017/8416763>
- Pratama, B.A., Susilowati, T., Yuniarti, T., 2018. Effect of temperature differences on incubation time, hatching rate, survival, and growth of gourami (*Osphronemus gouramy*) Bastar strain. *Jurnal Sains Akuakultur* 2(1), 59–65.
- Rao, K., 1995. Inhibition of DNA synthesis in primary rat hepatocyte cultures by malachite green: A new liver tumor promoter. *Toxicology Letters* 81, 107–113.
- Ramadhian, M.R., Soleha, T.U., Hanriko, R.,

- Azkiya, H.P., 2017. Effect of methanol extract of ketapang leaves (*Terminalia catappa* L.) on collagen fiber density in the healing of incision wounds in mice (*Mus musculus*). *ArgomedUnila* 4(1), 17–24.
- Rezinciuc, S., Sandoval-Sierra, J.V., Dieguez-Uribeondo, J., 2014. Molecular identification of a bronopol-tolerant strain of *Saprolegnia australis* causing egg and fry mortality in farmed brown trout (*Salmo trutta*). *Fungal Biology* 118(7), 591–600.
- Rustidja, 1997. Tropical fish hatchery techniques. Faculty of Fisheries, Brawijaya University, Malang.
- Saenal, S., Yanto, S., Amirah, A., 2020. Soaking eggs in ketapang leaf (*Terminalia catappa*) solution on the hatching rate of common carp (*Cyprinus carpio*) eggs. *Jurnal Pendidikan Teknologi Pertanian* 6(1), 115–124.
- Sarkar, P., Raju, V.S., Kuppasamy, G., Rahman, M.A., Elumalai, P., Harikrishnan, R., Arshad, A., Arockiaraj, J., 2022. Pathogenic fungi affecting fishes through their virulence molecules: A review. *Aquaculture* 548, 737553.
- Singh, S.P., Sharma, J.G., Ahmad, T., Chakrabarti, R., 2013. Effect of water temperature on the physiological responses of Asian catfish *Clarias batrachus* (Linnaeus 1758). *Asian Fisheries Science* 26, 26–38.
- Simanjuntak, F.J., Nirmala, K., Yuliana, E., 2021. Effect of recirculation systems on water quality, survival of gourami (*Osphronemus goramy*) juveniles, and business feasibility. *Pelagicus: Journal of Applied Fisheries and Marine Science* 2(1), 23–35.
- Srivastava, S., Sinha, R., Roy, D., 2004. Toxicological effects of malachite green. *Aquatic Toxicology* 66, 319–329.
- Sulistiyawati, D., Sri, M., 2009. Antifungal activity test of cashew leaf (*Anacardium occidentale* L.) infusion against *Candida albicans*. *Journal of Biomedicine* 2, 47–51.
- Susilo, N., Yusanti, I.A., 2022. Antifungal effectiveness of betel leaf extract (*Piper betle* L.) on the hatching rate and larval survival of gourami (*Osphronemus gouramy*). *Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam* 19(2), 175–185.
- Teletchea, F., Gardeur, J.-N., Kamler, E., Fontaine, P., 2009. The relationship between oocyte diameter and incubation temperature with incubation time in temperate freshwater fish species. *Journal of Fish Biology* 74(3), 652–668.
- Torto-Alalibo, T., Tian, M., Gajendran, K., Waugh, M.E., Van West, P., Kamoun, S., 2005. Expressed sequence tags from the oomycete fish pathogen *Saprolegnia parasitica* reveal putative virulence factors. *BMC Microbiology* 5, 46. <https://doi.org/10.1186/1471-2180-5-46>
- Triwardani, A., Basuki, F., Hastuti, S., 2022. Effect of soaking tawes fish (*Barbonymus gonionotus*) eggs in ketapang leaf (*Terminalia catappa*) solution on hatching rate. *Jurnal Sains Akuakultur Tropis* 6(2), 226–245.
- Yuniar, P., Subariyanto, Rivai, A.A., 2022. Effect of the combination of ketapang leaf (*Terminalia catappa*) and banana leaf (*Musa paradisiaca*) extracts on egg hatching and survival of betta fish (*Betta splendens*). *Jurnal Riset Akuakultur* 17(2), 71–84.

