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The effect of soaking duration in java cherry (*Muntingia calabura*) leaf extract on the hatching rate and survival of gourami (*Osphronemus gouramy*) larvae infected with *Saprolegnia* sp.

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Abstract
Gouramy fish is a high-value aquaculture commodity with substantial market demand and notable environmental adaptability. A primary challenge in its cultivation is the significant mortality rate of eggs and larvae, largely due to fungal infections caused by *Saprelognia* sp., leading to *Saprolegnia*sis. This infection is a key factor in reducing egg hatching rate (HR) and larval survival rate (SR). This study investigated the efficacy of cherry leaf (*Muntingia calabura*) extract, known for its antifungal properties, in mitigating this issue. The objective was to analyze the effect of different immersion durations in the extract on the HR and SR of gouramy larvae artificially infected with Saprolognia sp. A Completely Randomized Design (CRD) was employed with four treatments and three replications: A (0 minute), B (8 minute), C (12 minute), and D (16 minute). The observed parameters included fungal prevalence, HR, SR, and water quality. The results demonstrated that immersion in cherry leaf extract significantly (p<0.05) suppressed fungal prevalence and enhanced both HR and SR. The most effective treatment was the 12-minute immersion (Treatment C), which yielded the lowest fungal prevalence (17.78%), the highest HR (82.22%), and the highest SR (90.61%), while water quality parameters remained within the optimal range throughout the study. It is concluded that cherry leaf extract is an effective natural antifungal agent and is recommended for application on gouramy eggs with an immersion duration of 12 minutes.

Keywords: Aquaculture, hatching rate, *Muntingia calabura*, *Osphronemus goramy*, survival rate

1. Introduction
Aquaculture plays a pivotal role in Indonesia's fisheries sector, contributing significantly to national economic growth (Triani *et al*., 2023) [26]. One of the leading commodities in the freshwater aquaculture subsector is the gourami fish (*Osphronemus goramy*). Gourami is known for its high economic value in both local and international markets due to its delicious meat, high protein content, and adaptability to various environmental conditions (Sahara *et al*., 2023) [18]. Despite continuously increasing market demand, national gourami production over the past five years has shown instability. Data from the Ministry of Marine Affairs and Fisheries (2024) records production fluctuations: 152,668 tons (2020), 149,169 tons (2021), 159,571 tons (2022), 153,939 tons (2023), and 196,675 tons (2024). The high market demand for gourami makes the hatchery aspect a critical factor in supporting sustainable production. Unfortunately, this increase in demand is not balanced with production output, partly because gourami growth is relatively slow, primarily due to high mortality rates at the egg and larval stages (Suwarsito *et al*., 2024) [25]. Previous studies using plant extracts like bay leaf and ketapang have shown limited effectiveness, yielding HR below 52% (Hastuti *et al*., 2022) [9]. This highlights the need for more potent bioactive sources. Cherry leaf (*Muntingia calabura*) is a promising candidate, containing high levels of antifungal compounds such as tannins (13.715%) and flavonoids (13.375%), significantly higher than those found in bay leaves (Soraya, 2023) [22]. Preliminary evidence suggests cherry leaf extract can increase HR beyond 70% and larval survival rate (SR) to 80%

(Sahi *et al*., 2023) [19].
Therefore, this study aims to determine the effect of immersion duration in cherry leaf extract on the HR and SR of *Saprolegnia*-infected gourami eggs and larvae. The findings are expected to provide an effective, eco-friendly solution to support sustainable gourami production.
The hypothesis used in this study is to determine whether there is an effect of immersion duration in cherry leaf (*M. calabura*) extract on the hatching rate and survival rate of gourami (*O. gouramy*) larvae infected with *Saprolegnia* sp.

- **H_0:** The immersion duration in cherry leaf extract has no significant effect on the hatching rate and survival rate of gourami larvae infected with *Saprolegnia* sp.
- **H_1:** The immersion duration in cherry leaf extract has a significant effect on the hatching rate and survival rate of gourami larvae infected with *Saprolegnia* sp.

2. Materials and Methods

The materials utilized in this study included 720 test eggs of gourami (*O. gouramy*) obtained from the Freshwater Aquaculture Center in Muntilan. The eggs were stocked at a density of 60 eggs per liter per treatment replicate, A total of 36 ml of cherry leaf (*M. calabura*) extract was prepared, with each treatment application using 3 ml of the extract. The water medium was sourced from a natural spring at the research location. The experimental setup employed 12 glass jars for egg immersion and 12 fourteen-liter buckets as hatching containers. An aerator system was installed to maintain oxygen levels during incubation. Water quality parameters were monitored using a thermometer for temperature measurement, a pH meter for acidity levels, and a DO meter for dissolved oxygen concentration. Egg transfer and counting were facilitated using tablespoons, while larval handling was accomplished with fine mesh nets. Documentation was performed using a digital camera, and all observations were recorded using standard stationery.
The study employed a Completely Randomized Design (CRD) with four treatments and three replications. The treatments consisted of varying immersion durations in cherry leaf extract:
Treatment A (0-minute immersion), Treatment B (8-minute immersion), Treatment C (12-minute immersion), and Treatment D (16-minute immersion). Each treatment container was stocked with 60 eggs per liter of water, following standardized aquaculture protocols for egg incubation.

2.1 Research Procedure

a. Preparation of Experiment Tamks

Tanks for immersing gouramy fish eggs in Muntingia calabura leaf extract solution consisted of twelve 8 L units (21 cm height, 20 cm diameter). Twelve 14 L tanks were prepared for egg incubation and larval rearing. All tanks were cleaned with a sponge and soap, rinsed thoroughly with running water, and air-dried. The incubation buckets were filled with water to half their height. An aerator was installed in each incubation tank to supply oxygen. All containers were labeled for identification.

b. Extract Preparation

The *M. calabura* leaf extract was prepared using a maceration method. One hundred grams (100 g) of leaf powder was extracted with 750 ml of 96% ethanol, following the procedure of Puspitasari *et al*. Ethanol was selected for its ability to extract both polar and non-polar compounds. After maceration, the mixture was filtered, and the filtrate was concentrated using a rotary evaporator to obtain a thick extract.

c. Phyrocemical Screening

A preliminary phytochemical analysis was conducted on the *M. calabura* leaf extract to identify the presence of secondary metabolites, following the method of Natasha *et al*. The tested compounds included flavonoids and tannins.

d. Fungal Identify

Saprolegnia sp. isolates were obtained from infected gouramy eggs. The fungus was cultured on Potato Dextrose Agar (PDA) and incubated for three days, following the method of Yasani *et al*. (2024) [29]. Fungal identification was performed at Cendekia Harmoni Laboratory, Semarang, through macroscopic observation of colony morphology and microscopic examination of hyphal structures at 150x magnification.

e. Fungal Infection

Gouramy eggs were obtained from the Muntilan Freshwater Fish Breeding Center. To induce infection, 40 healthy eggs were co-cultured with 10 fungus-infected eggs in a single tank for 6 hours (one day), simulating natural transmission, as described by Tahya *et al*. Laboratory identification confirmed the infecting fungus as *Saprolegnia* sp.

f. Eggs immersion in extract

The gouramy eggs were immersed in the M. calabura leaf extract at a concentration of 1.5 ml/L, a dosage previously reported by Sahi *et al*. (2023) [19] to yield optimal results for growth and survival in common carp larvae. After treatment, the eggs were transferred to aerated incubation tanks. The number of successfully hatched larvae in each treatment was recorded.

2.2 Measurement Variables

2.2.2 Fungal Identification and Prevalence

Fungal infection was identified visually and confirmed microscopically. Infected eggs exhibited cotton-like fungal growth. Following the methodology of Andika *et al*. (2014) [3] Prevalence was calculated as:

$$\text{Prevalence (\%)} = \frac{\text{Number of infected eggs}}{\text{Total number of eggs}} \text{ x } 100\%$$

2.2.3 Observation of Embryonic Development

Embryonic development was monitored microscopically by placing eggs on concave glass slides. Critical developmental stages including one-cell, two cell, four cell, multi cell, morula, blastula, gastrula, and organogenesis were documented according to Murjani *et al*. (2021) [16]. Hatching typically occurred within 30-37 hours post-fertilization

2.2.4 Hatching Rate of Gourami Fish Eggs

The hatching rate (HR) of gourami fish eggs was determined by calculating the percentage of successfully hatched eggs relative to the total number of fertilized eggs, following the methodology of Amelia *et al*. (2024). The formula used was:

$$HR\ (\%) = \frac{\text{Number of hatched eggs}}{\text{Total number of eggs}} \times 100\%$$

2.2.5 Survival Rate of Gourami Fish Larvae

The survival rate (SR) of larvae was evaluated by measuring the percentage of larvae that survived until the end of the 10-day rearing period, as described by Widiastuti (2009) [28]. The calculation was performed using the formula:

$$SR(\%) = \frac{N_t}{N_0} \times 100\%$$

Where Nt represents the number of larvae at the end of rearing and No denotes the number of larvae at the start of rearing.

2.2.6 Water Quality

Water quality parameters, including temperature (°C), pH, salinity (ppm), and dissolved oxygen (DO, mg/L), were monitored to maintain optimal conditions for gourami egg incubation and larval survival. Temperature was measured using a thermometer, pH with a pH meter, and dissolved oxygen with a DO meter. Measurements were conducted twice daily (morning and evening) following established protocols
(Endar *et al*., 2015) [4].

2.3 Data Analysis

Data from the research results will be carried out using normality test and homogeneity test. Then, an analysis was conducted using ANOVA (Analysis of Variance). If the ANOVA test results indicate a significant difference among the treatments, it will be continued with the DMRT (Duncan Multiple Range Test).

3. Results

3.1 Phytochemical Test

The results of the phytochemical test on kersen leaf (*Muntingia calabura*) extract, based on the research conducted are presented in table 1.

Table 1: Phytochemical Test Results of Kersen Leaves

Compound	Replication 1	Replication 2	Average
Flavonoids (%)	1,0413	1,0196	1,0335
Tannins (%)	3,5095	3,5131	3,5113

In the tannin test, values of 3.5095% and 3.5131% were obtained for the first and second replications, respectively, with an average of 3.5113%. Meanwhile, the flavonoid content was 1.0413% in the first replication and 1.0196% in the second, averaging 1.03345%. The values obtained in this study indicate that the kersen leaf extract contains a sufficient amount of flavonoids to support its potential application in preventing fungal growth on gourami fish eggs.

3.2 Fungal Identification and Prevalence

Microscopic observation of the isolate obtained from gourami fish eggs showed characteristic structures leading to identification as *Saprolegnia* sp. In general, the observed hyphae appeared broad, aseptate, and branched with transparent walls. This hyphal shape is a primary characteristic of the Oomycota group. This condition shows that the isolate grown on the culture medium has developed according to the general characteristics of water molds. The results of the observed *Saprolegnia* images under the microscope can be seen in figure 2.

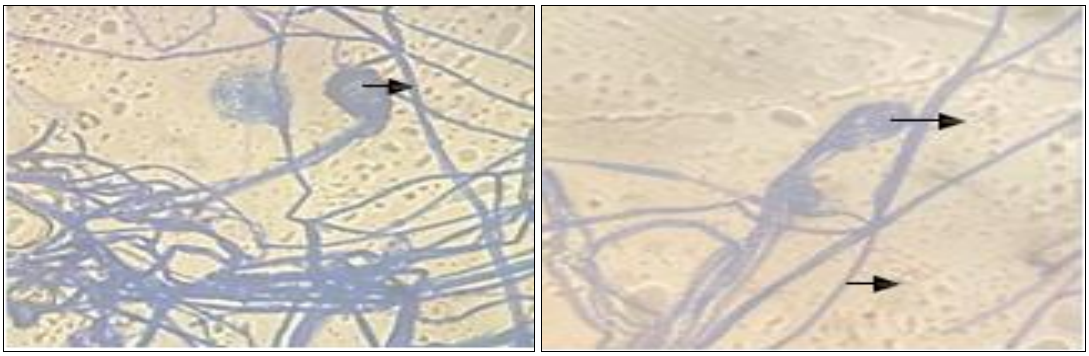


Fig 1: Microscopic Structure of *Saprolegnia* sp. Description: (A) Oogonium (1) at 150x magnification, (B) Sporangium (2) and aseptate hyphae (3) at 150x magnification.

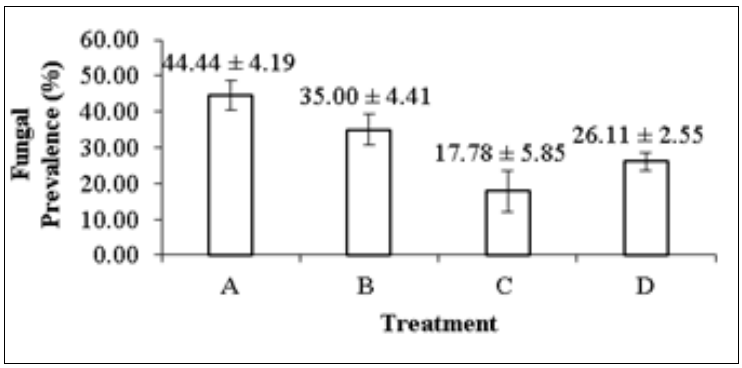


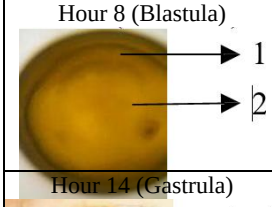
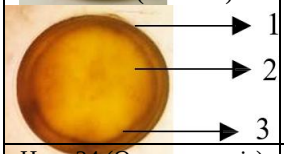
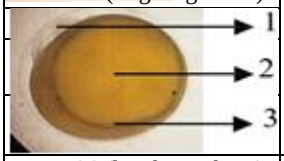
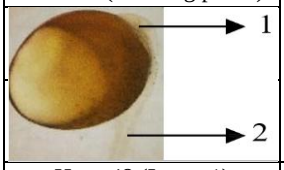
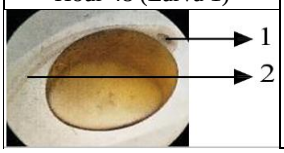
Fig 2: Fungal Prevalence on Gourami Eggs Description: Treatments: A (0 minutes), B (8 minutes), C (12 minutes), D (16 minutes). Means with different superscript letters are significantly different according to Duncan's multiple range test (P<0.05)

The fungal prevalence on gourami eggs shows differences due to immersion in kersen leaf extract with varying durations. The highest fungal prevalence was observed in treatment A (0 minutes) at 44.44±4.19%, while the lowest fungal prevalence was found in treatment C (12minute immersion) at 17.78±5.85%. The normality and homogeneity tests on the fungal prevalence data indicated that the data are normally distributed and homogeneous. Subsequently, after conducting an analysis of variance, the results showed that the fungal prevalence on gourami eggs indicated that the immersion in kersen leaf extract with varying durations had a significant effect (P<0.05); therefore, we reject H_0 and accept H_1. The results of the Duncan test showed that treatment A is significantly different from treatments B, C, and D at the 5% level (P<0.05). Furthermore, treatment B is significantly different from treatments C and D at the 5% level (P<0.05), and treatment C is significantly different from treatment D at the 5% leve l (P<0.05).

3.3 Embryonic Development of Gourami

The observation of gourami egg development following immersion in kersen leaf extract yielded the following results, as presented in the table 2.

Table 2: Embryonic developmental stages of gourami (*Osphronemus goramy*)

Embryogenesis Phase	Morphological Description
Hour 8 (Blastula) 	1. 1. Blastoderm 2. 2. Yolk
Hour 14 (Gastrula) 	1. Corion 2. Yolk 3. Embryonic shield
Hour 24 (Organogenesis) 	1. Notchord 2. Yolk 3. Embryonic shield
Hour 36 (hatching phase) 	1. Head bud 2. Somites
Hour 48 (Larva 1) 	1. Eye 2. Vertebral column

3.4 Hatching Rate (HR)

The calculation of the number of hatched gourami eggs was conducted after immersion in kersen leaf (*Muntingia calabura*) extract with varying immersion times. The research yielded hatching rate results presented in Figure 3.

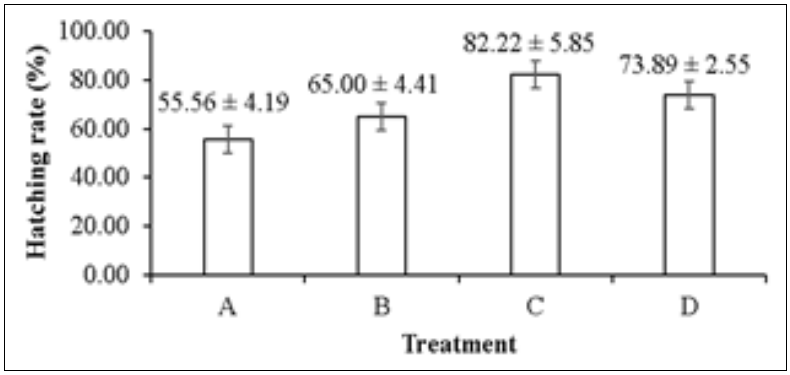


Fig 3: Hatching Rate on Gourami Eggs Description: Treatments: A (0 minutes), B (8 minutes), C (12 minutes), D (16 minutes). Means with different superscript letters are significantly different according to Duncan's multiple range test (P<0.05)

The hatching rate of gourami eggs shows differences due to immersion in kersen leaf extract with varying durations. The highest hatching rate was observed in treatment C (12 minute immersion) at 82.22±5.85%, while the lowest hatching rate was found in treatment A (0 minutes) at 55.56±4.19%. The normality and homogeneity tests on the hatching rate data indicated that the data are normally distributed and homogeneous. Subsequently, after conducting an analysis of variance, the results showed that the hatching rate of gourami eggs indicated that the immersion in kersen leaf extract with varying durations had a significant effect (P<0.05); therefore, we reject H_0 and accept H_1. The results of the Duncan test showed that treatment C is significantly different from treatments D, B, and A at the 5% level (P<0.05). Furthermore, treatments B and D are not significantly different from each other, nor are treatments B and A, but both were better than treatment D. Treatment D had the lowest value and was significantly different from treatments C, B, and A at the 5% level (P<0.05). Thus, the best result was obtained in treatment C with a 12 minute immersion time.

3.5 Survival Rate (SR)

Based on the research, larval survival rate was observed for 10 days post-hatching. Observation involved counting the remaining larvae over 10 days of rearing. The obtained SR data are shown in Figure 4.

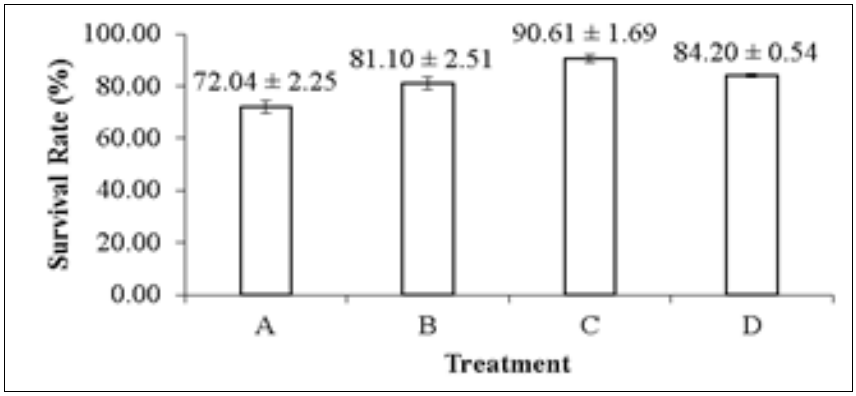


Fig 4: Survival Rate on Gourami Larval Description: Treatments: A (0 minutes), B (8 minutes), C (12 minutes), D (16 minutes). Means with different superscript letters are significantly different according to Duncan's multiple range test (P<0.05)

The survival rate of gourami eggs shows differences due to immersion in kersen leaf extract with varying durations. The highest survival rate was observed in treatment C (12 minute immersion) at 90.61±1.69%, while the lowest survival rate was found in treatment A (0 minutes) at 72.03±2.25%. The normality and homogeneity tests on the survival rate data indicated that the data are normally distributed and homogeneous. Subsequently, after conducting an analysis of variance, the results showed that the survival rate of gourami eggs indicated that the immersion in kersen leaf extract with varying durations had a significant effect (P<0.05); therefore, we reject H0 and accept H1. The results of the Duncan test showed that treatment C is significantly different from treatments D, B, and A at the 5% level (P<0.05). Furthermore, treatment D is significantly different from treatments B and A at the 5% level (P<0.05), and treatment B is
different from treatment A at the 5% level (i<0.05).

3.6 Water Quality

The quality of water measured during the research includes data on temperature, dissolved oxygen (DO), and pH. These water quality measurements were conducted twice daily, in the morning and afternoon. The results of the water quality parameter measurements for the hatching of gourami fish eggs are presented in Table 1.

Table 1: Water Quality of *Diaphanosoma* sp.

Variables	A	B	C	D	Reader*
Temperature (°C)	26,1-29,4	26,0-29,5	26,2-29,4	26,0-29,4	24-30[a]
DO (mg/L)	5,6-7,3	5,4-7,4	5,5-7,3	5,5- 7,4	<5[a]
pH	7,1-7,5	7,1-7,5	7,1-7,5	7,1-7,5	4-8b

Description: a) SNI 01-7241-2006, b) Sugihartono *et al*, (2017) [23]

Based on the measurement results, the water quality parameters
(temperature, DO, and pH) of the gourami egg hatching medium throughout the study were consistently within the optimal range suitable for embryogenesis and larval survival. Thus, the achieved water quality conditions have supported the successful hatching process and larval rearing of gourami in this study.

4. Discussion

4.1 Phytochemical Test

The phytochemical test results showed that kersen leaf (*M. calabura*) extract contains bioactive compounds, especially tannins (average 3.5113%) and flavonoids (average 1.03345%), which have the potential to enhance hatchability, survival, and prevention of *Saprolegnia* infection in gourami eggs. Tannins are known for their astringent properties, which can strengthen the egg membrane, reduce pathogen penetration, and increase embryo resistance to environmental

stress. The relatively high tannin content in this extract is suspected to form a protective layer on the egg surface while inhibiting the growth of pathogenic microbes that disrupt embryonic development. Meanwhile, flavonoids act as antioxidants, protecting the eggs from free radicals and oxidative stress during incubation, thus supporting embryo survival. The average flavonoid content of 1.03345% also indicates that kersen leaves contain bioactive compounds involved in antioxidant and antimicrobial activities. Flavonoids work by inhibiting bacterial growth through membrane destabilization and interference with microbial metabolism. These results align with previous research reporting that flavonoids in M. calabura contribute to its pharmacological effects, including anticancer and hepatoprotective activities (Handayani *et al*., 2024) [8]. Based on the phytochemical analysis, kersen leaf extract showed significantly higher bioactive compound content compared to salam leaf (*Syzygium polyanthum*). Kersen leaves had an average tannin content of 35.113 mg/g, while salam leaves contained only 0.854 mg/g (Soraya, 2023) [22]. The average flavonoid content in kersen leaves was 10.3345 mgQE/g, compared to 6.089 mgQE/g in salam leaves (Hastuti *et al*., 2022) [9].

4.2 Fungal Identification and Prevalence

In this study, microscopic observation of the isolate obtained from gourami eggs showed characteristics consistent with *Saprolegnia* sp. This organism belongs to the Oomycota group, water molds commonly found in freshwater environments and known as important pathogens for fish and fish eggs. According to Kadir *et al*. (2023) [13], *Saprolegnia* sp. is often associated with *Saprolegnia*sis, which can cause mass mortality in the embryonic and larval stages of fish. This is due to the ability of *Saprolegnia* sp. hyphae to grow invasively on host tissues, disrupting embryonic development. Observation of the *Saprolegnia* sp. structure was conducted microscopically using Lactophenol Cotton Blue stain. Fungal colonies were observed under a microscope at 150x magnification. The observation results showed characteristics consistent with the typical features of the genus *Saprolegnia* sp. The presence of broad, transparent, aseptate hyphae was a key distinguishing marker from other fungal groups. The hyphae of *Saprolegnia* sp. lack septa but branch extensively, forming a dense mycelium. This aligns with the statement by, that *Saprolegnia sp.* has slender, smooth, aseptate vegetative hyphae with moderate branching.

Furthermore, cylindrical to round sporangia located at the hyphal tips were observed, containing numerous biflagellate zoospores for dispersal. Some observations also revealed round, thick-walled oogonia containing oospores, which represent the characteristic sexual reproduction phase of Saprolegnia sp. This combination of asexual and sexual characteristics strengthens the evidence that the isolate found indeed belongs to the genus Saprolegnia. This is consistent with the explanation by Lone (2018) [15] that the life cycle of Saprolegnia sp. occurs through both asexual and sexual reproduction. In the asexual phase, sporangia produce zoospores that can swim freely using flagella, facilitating spread and finding new hosts. In the sexual phase, oogonia (female) and antheridia (male) are formed, which then unite to produce thick-walled oospores as a survival structure.

Fish eggs have a natural susceptibility to aquatic fungal infections. This fungus primarily infects unfertilized or dead eggs. However, the infection can potentially spread and attack healthy eggs. Essentially, almost all types of fish eggs are vulnerable to this fungus. The high fungal infection observed in treatment A (control) is suspected because the eggs did not receive immersion in kersen extract. Consequently, the eggs lacked protection from the antifungal compounds in the extract. This condition triggered uncontrolled fungal growth, which then spread and attacked healthy eggs, causing mortality and hatching failure. Meanwhile, treatment C (12 minutes) showed the lowest level of Saprolegnia infection. This is attributed to the antifungal compounds contained in kersen leaves. These active compounds, including flavonoids and tannins, are suspected to effectively inhibit fungal growth. This is supported by the statement of Kurniawati *et al*. (2016) [14] that kersen leaves contain flavonoids and tannins effective as antifungals. Flavonoids work by damaging the structure of the fungal cell membrane through their lipophilic properties, causing cell leakage and protein denaturation. Meanwhile, tannins inhibit chitin synthesis in the fungal cell wall, disrupting fungal growth and development. This combination of mechanisms makes kersen leaf extract capable of significantly suppressing fungal infection.

4.3 Embryonic Development of Gourami

Embryogenesis is the process of embryo formation and development in fish. This process involves cell development stages after fertilization. Observation of gourami egg development was conducted after fertilized eggs were collected from the nest. The collected eggs were then immersed in kersen leaf extract according to the predetermined dosage and duration. Development was observed for each treatment using a microscope. Based on observations, the embryonic development of gourami (O. gouramy) proceeded rapidly and clearly followed a consistent pattern under optimal temperature conditions. At 8 hours post-fertilization (hpf), the embryo entered the early gastrula stage, marked by the formation of the germ ring due to epiboly of the blastoderm cells (Had *et al*., 2022) [7]. This structure initiates the formation of the body axis and embryonic tissue differentiation. This process continued until 14 hpf towards the late gastrula stage, where cells covered more than 70% of the yolk surface, and the formation of the three primary germ layers (ectoderm, mesoderm, endoderm) was completed. Cell invagination during this phase is crucial for subsequent embryonic development.

The organogenesis phase began at 21 hpf, where the differentiation of body organs such as the nervous system, eyes,

and somites became clearly observable. At this stage, the heart precursor began beating, and optic vesicles appeared as two dark spots on either side of the head. The embryo hatched at 36 hpf, driven by increasing larval activity and the secretion of choriolytic enzymes that soften the eggshell. Increased water temperature and dissolved oxygen levels also contributed to triggering the hatching process. After hatching, at 48 hpf, the larvae entered the larval stage 1, characterized by total dependence on the yolk sac and limited, unstable movement (Gunawan *et al*., 2024) [6]. Larvae typically exhibited brief vertical swimming behavior and spent more time resting at the bottom of the container. This phase is a critical adaptation period where the respiratory and digestive systems begin to function independently.

4.4 Hatching Rate (HR)

Hatching rate (HR) is a critical parameter in gourami culture

as it directly determines the number of seeds produced. A high hatching rate not only reflects the success of the hatching process but also indicates egg quality and optimal rearing environmental conditions (Hidayah *et al*., 2021) [10]. The research results showed that immersing gourami eggs in kersen leaf extract with varying durations significantly affected the hatching rate (HR) of the gourami eggs. The study on immersing gourami eggs in kersen leaf solution showed variation in the hatching percentage based on immersion time. The highest result was obtained in treatment C (82.22±5.85%) with a 12-minute duration, followed by treatment D (73.89±2.55%) for 16 minutes, and treatment B (65.00±4.41%) for 8 minutes. Meanwhile, treatment A with 0 minutes immersion only reached 55.56±4.19% hatchability. This study indicates that the duration of immersion in kersen leaf extract significantly influences the hatching success of gourami eggs, with an optimal time of 12 minutes.

Hatching rate (HR) is a critical indicator of successful fish breeding, influenced by the complex interaction between internal (egg quality, genetics) and external (environment, pathogens) factors. Observations on the hatching rate (HR) showed variation in the percentage of hatched eggs across treatments. Furthermore, immersion in kersen leaf extract also indicated that kersen leaves are suspected to prevent fungal attacks, characterized by the appearance of milky white threads and cloudy color in infected eggs. The high hatching rate in the 12 minute immersion treatment is suspected to be related to the optimization of bioactive compound absorption from the protective kersen leaf extract on the embryo. The flavonoid and tannin compounds contained in the extract are suspected to play a role in suppressing pathogenic microorganism growth while providing a more conducive environment for embryonic development (Mustofa *et al*., 2022) [17]. Conversely, the decrease in hatching rate in the 16-minute treatment indicates that excessively long immersion duration may have caused physiological stress on the embryo, consistent with research on sangkuriang catfish eggs which showed that longer immersion duration in extract can negatively impact hatching rate (Tumanggor *et al*., 2023) [27]. Meanwhile, the low hatching rate in treatment A shows egg vulnerability to environmental conditions without bioactive compound protection. Untreated eggs were more easily infected compared to those immersed in kersen leaf extract. This is because the treated eggs have a protective layer of antifungal substances, making them less susceptible. Hyphae attached to the eggs can weaken the strength of the egg chorion (Sumahiradewi *et al*., 2022) [24].

Kersen leaf extract showed a positive effect on egg hatching through the antifungal activity of its tannin and flavonoid content. These two compounds work synergistically to inhibit the growth of Saprolegnia sp., the primary pathogen causing fish egg mortality. The mechanism of flavonoids as antimicrobials involves damaging the function of the fungal membrane and cell wall, while the antimicrobial properties of tannins affect the biosynthesis process related to cell wall and membrane synthesis, so that changes in membrane permeability can reduce cell volume.

4.5 Survival Rate (SR)

Based on the research results, it was shown that immersing gourami eggs in kersen leaf extract with varying immersion durations significantly affected the survival rate of gourami larvae. Observations of gourami larval survival were conducted for 10 days, starting from egg hatching until the larvae stage. The 10-day post-hatching observations revealed that treatment A (0minute immersion) had the lowest survival rate (72.04±2.25%), compared to treatment B (81.10±2.51%), treatment D (84.20±0.54%), with treatment C showing the highest percentage (90.61±1.69%). The surviving fish are suspected to possess natural body resistance and are influenced by the active compounds in kersen leaf extract that enhance fish immunity. Based on statistical analysis results, there was a significant difference between the 0 minute immersion treatment and all other immersion treatments, indicating that kersen leaf extract not only functions as an antimicrobial but also acts as a natural immunostimulant. This aligns with the research by Sahi *et al*. (2023), who found that phenolic compounds in kersen leaves can enhance the physiological resistance of fish.

The low survival rate in treatment A (72.04±2.25%) indicates that without the protection of kersen leaf extract, larvae are more susceptible to pathogen infections and environmental stress. The high survival rate of gourami larvae in treatment C is suspected because kersen extract can protect the fish's immune system. This is attributed to tannins in kersen leaves playing a role in increasing the efficiency of enzymes involved in the hatching process of gourami eggs (*Osphronemus goramy*). The high larval survival rates in treatments B, C, and D demonstrate that kersen leaf extract can act as an immunostimulant that enhances the physiological resistance of gourami larvae (Sahi *et al*., 2023) [19].

Flavonoids work by permanently damaging the protein structure and cell membranes of bacteria, making the bacteria unable to repair the damage. Tannins in kersen leaf extract function to increase the efficiency of crucial enzymes during the hatching process and early larval development, while flavonoids work by irreversibly damaging the protein structure and cell membranes of pathogenic microorganisms. This mechanism corresponds with the findings of Fadel *et al*. (2021) [5], who reported the effectiveness of flavonoids in inhibiting various pathogenic microorganisms. These findings strengthen the potential of kersen leaf extract as a natural alternative in gourami cultivation, particularly during the critical phases of hatching and larval development. However, further research is needed to determine optimal parameters such as the most effective concentration and immersion duration on a larger production scale.

4.6 Water Quality

Water quality parameters critical for gourami (*O. gouramy*) egg incubation temperature, dissolved oxygen (DO), and pH were monitored twice daily throughout the study. The measured values, with temperature ranging from 26.0 to 29.5°C, fell within the optimal range for gourami embryonic development (Simanjuntak *et al*., 2021) [21]. Similarly, dissolved oxygen levels (5.4-7.4 mg/L) and pH (7.1-7.5) were maintained within suitable ranges for larval rearing, although extreme pH levels can induce mortality in early life stages (Irawan *et al*., 2021) [11]. The stability of these parameters was crucial for isolating the specific effects of the kersen leaf extract treatments on embryonic development and larval survival. Maintaining optimal water quality is fundamental for successful hatchery operations. Temperature directly regulates metabolic and enzymatic activity during embryogenesis, while adequate DO is indispensable for respiration and energy

metabolism (Jumaidi *et al*., 2017) [12]. Consistent aeration was provided to sustain oxygen levels, as oxygen deficiency can suppress feed conversion and growth while increasing disease susceptibility (Jumaidi *et al*., 2017) [12]. Furthermore, stable pH conditions are crucial for maintaining physiological homeostasis and minimizing stress in developing larvae (Simanjuntak *et al*., 2021) [21]. The maintained water parameters in this study effectively supported the incubation environment, ensuring that observed results could be attributed to experimental treatments rather than environmental variability.

5. Conclusion

Based on the results of the research that has been conducted that kersen leaf (*M. calabura*) extract, containing bioactive compounds tannins (3.5113%) and flavonoids (1.03345%), significantly improves gourami (*O. gouramy*) hatchery performance. The optimal 12 minute immersion treatment yielded the best results with fungal prevalence of 17.78%, hatching rate of 82.22%, and larval survival rate of 90.61%. The extract's efficacy is attributed to the synergistic antifungal action of its compound's flavonoids damage fungal cell membranes while tannins inhibit chitin synthesis. Under optimal water quality conditions (temperature 26.0-29.5°C, pH 7.1-7.5, DO 5.4-7.4 mg/L), kersen leaf extract proves to be an effective natural alternative for controlling *Saprolegnia* sp. infection and enhancing larval viability in gourami cultivation.

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