The effect *Calotropis gigantea* leaf extract on eggs hatchability and survival of *Barbonymus gonionotus* larvae

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**Abstract.** This study aims to determine the *Calotropis gigantea* extract against hatchability of *Barbonymus gonionotus* eggs infected with pathogenic fungi. The research was conducted at Fish Seeds Center Krueng Batee, Aceh Barat Daya district, from July to August 2020. Statistical analysis used a completely randomized design (CRD) method with 6 treatment and 3 replications for each treatment. The treatments were carried out by immersing the *Barbonymus gonionotus* eggs into *C. gigantea* leaf extract. Namely Control (without extract), A (400 ppm extract), B (800 ppm extract), C (1200 ppm extract), D (1600 ppm extract), and E (2000 ppm extract). ANOVA test results showed that the addition of *C. gigantea* leaf extract had a significant effect (P<0.05) on hatchability and survival of *B. gonionotus* larvae. The best treatment was achieved at the C treatment (800 ppm extract) with 88 % hatching rate and 92.54 % survival rate. From the results of this study, it is known that using *C. gigantea* leaf extract can increase the hatchability of *B. gonionotus* fish eggs infected with the *Saprolegnia* sp. pathogen, which means it can also increase the production of fish seeds.

1. Introduction

One type of freshwater fish that has economic value and is favored by people in the Aceh area is *Barbonymus gonionotus* [1,2]. The development of fish farming has been carried out, but has problems in terms of procuring seeds. *B. gonionotus* hatchery process is constrained, among others, less successful in the process of hatching eggs which will eventually have a further impact on the provision of fish seeds. Alfath et al. [3] said that the egg hatching process is one of the phases that determine the success of fish farming. High egg hatchability with good egg quality will make the production of *B. gonionotus* eggs is infection with pathogens from the fungal group. The type of fungus that often infects fish eggs is...
Saprolegnia sp. This is in accordance with the statement of Lingga et al. [4] that one of the fungal infections that often attacks fish and fish eggs is the fungus Saprolegnia sp., so that it can reduce the degree of hatching of eggs. According to Rezinciuc et al. [5] Saprolegniasis disease can cause considerable economic losses in aquaculture. Furthermore, Magray et al. [6] said that Saprolegnia spp poses a serious threat to the welfare and resilience of the sishing industry, thus potentially causing severe socio-economic losses for stakeholders involved in trade.

The prevention of fungal infections in fish, in order to avoid the use of antibiotics which are believed to be unfriendly to the environment and humans, is to use herbal plants. Zuraidah dan Silkhairi [7] stated that the use of traditional Saprolegnia sp. Several studies have been conducted on the use of herbal plants to prevent or treat the fungal disease Saprolegnia sp. on fish eggs, namely the use of flower extract of Nicolia speciosa Horan [8] and leaf extract Piper betle L [9]. Another herbal plant that can be used to treat pathogenic diseases in fish eggs is extract of Calotropis gigantea leaves. C. gigantean is a plant that has many uses, both from the leaves, stems, or roots. The chemical constituents of C. gigantea leaves include flavonoids, steroids, saponins, alkaloids and tanins [10]. The results of research conducted by Mulya [11] using C. gigantea leaves extract were able to increase the hatchability of peres fish eggs at a concentration of 1200 ppm (87.75%) and were also very effective in inhibiting the spread of fungi that generally attack fish eggs peres in an unfavorable environment. Considering that one of the obstacles to hatching B. gonionotus eggs in aquaculture activities is infection with the fungus Saprolegnia sp., the researchers studied the effect of soaking C. gigantea leaves extract on the hatchability of fish eggs. This study aimed to examine the effect of C. gigantea leaves extract on hatchability of B. gonionotus eggs infected with fungal pathogens.

2. Material and Methods

2.1. Tools and material

The tools used in this research are Vacuum Rotary Evaporator, blender, picnometer, bucket plastic, aerators, mikroscope, beaker glass, filter paper, pipette drops, DO meters, thermometers, pH meters. The material used are B. gonionotus eggs, C. gigantea leaves, Saprolegnia sp. and ethanol.

2.2. Method

The study was conducted at Fish Seeds Center Krueng Batee, Aceh Barat Daya district, Aceh Province Indonesia at August 2020. The design of this study is to use a completely randomized design (CRD) using 6 treatments and 4 replications. The treatments tested in this study are as follows:

- Control = soaking without C. gigantea leaves extract (0 ppm)
- A = soaking with C. gigantea leaves extract dose of 400 ppm
- B = soaking with C. gigantea leaves extract dose of 800 ppm
- C = soaking with C. gigantea leaves extract dose of 1200 ppm
- D = soaking with C. gigantea leaves extract dose of 1600 ppm
- E = soaking with C. gigantea leaves extract dose of 2000 ppm

2.3. Calotropis gigantea leaves extract

Calotropis gigantea leaves were dried at room temperature for approximately four days under supervision. The dried samples (simplicia) were cut into pieces and then mashed using a blender to become simplicia powder. The simplicia powder was weighed as much as 500 g and put into a container. Then it was soaked (maceration) with 4 L of 95% absolute ethanol solution and soaked for 24 hours. After 24 hours, the solution was filtered using a sieve and evaporated using an evaporator. Furthermore, the extract was weighed for its specific gravity and stored in the freezer until it was used for testing. In accordance with the level of treatment [12].
2.4. Fish eggs soak
The sample used in this study was *B. gonionotus* eggs obtained from spawning in a pond at the Kreung Batee Fish Seed Center, Southwest Aceh. Furthermore, as many as 25 eggs that have been infected with fungi are put into each container (jar) containing 100 healthy eggs and installed an aerator. Healthy eggs are left to soak for 6 hours until they become infected with fungus. Then the healthy eggs that had been infected with the fungus were immediately transferred into each container that had been given a solution of thistle leaf extract according to the concentration specified for each treatment. Soaking in a solution of thistle leaf extract is carried out for 20 minutes. Furthermore, for the observation of eggs recovered from fungal infections using a digital microscope with an hourly interval, then the development of eggs was photographed as documentation. The eggs that have been observed are returned to the container and their development is observed until they hatch. Fish larvae that have hatched are observed for survival for 14 days.

2.5. Observed parameters

2.5.1. Eggs condition
Eggs were observed starting after being soaked using a solution of *C. gigantea* leaves extract until the eggs hatched, both dead eggs (infertile) and infected eggs. Eggs that are attacked by fungus will show signs around the eggs that there are threads that cover the eggs like cotton, observation of eggs that are attacked by fungus is done visually or with the help of a light microscope.

2.5.2. Identification of Fungi on eggs
Observations on eggs that were attacked by fungi using a microscope referred to the characteristics of fungi stated by Webster and Weber [13] that *Saprolegnia* sp. has hyphae that is senisitic, not insulated, branched and at the end of the pipe there is a zoosporangium containing zoospores. Eggs that are attacked by fungus are characterized by the growth of fine threads resembling cotton on the surface of the eggs [14].

2.5.3. Hatching rate (HR)
The hatching rate was calculated according Arfiah et al. [15]:

\[
HR = \frac{\text{Number of eggs that hatch}}{\text{Number of fertilized eggs}} \times 100\%
\]

Where: HR = Hatching Rate (%)

2.5.4. Survival rate
The survival rate was calculated according Effendie 16):

\[
SR = \frac{No - Nt}{No} \times 100
\]

Where: SR = Survival Rate (%); Nt = number of dead fish (ind); No = number of fish observed (ind).

2.5.5. Water quality parameters
The observed water quality was temperature, pH and dissolved oxygen. Temperature was measured using a thermometer, pH was measured using a pH meter, and dissolved oxygen was measured using a DO meter. Water quality is measured every 06.00 and 12.00 AM.

2.6. Data analysis
Analysis of research data using Analysis of Variance (ANOVA). If the results have a significant effect, it will be continued with Duncan’s test with a 95% confidence level. Water quality parameters were analyzed descriptively.
3. Results and Discussion

*C. gigantea* leaf extract was effective in suppressing fungal growth on fish eggs. The results of the ANOVA test of the research data showed that the immersion of the leaf extract of *C. gigantea* had a significant effect on egg hatchability and survival of *B. gonionotus* larvae infected with fungi (P<0.05) (Table 1). Based on observations, it was known that *C. gigantea* leaves were able to inhibit the growth of fungi on *B. gonionotus* fish eggs. This is presumably because the *C. gigantea* plant contains a variety of chemical compounds that can be used as antifungal agents. Several studies have shown the presence of active chemical compounds in parts of the leaf plant of *C. gigantea* such as the content of flavonoids, steroids, tannins, phenols and alkaloids as well as saponins [17,18,19]. Tannin compounds function as antibacterial, containing active compounds that can kill microbes by damaging the membrane. Flavonoids have the ability to form complexes with proteins and damage fungal cell membranes by denaturing protein bonds in cell membranes, so that cell membranes become lysed and these compounds penetrate into the cell nucleus and cause damage to fungal cell tissue so that fungi cannot grow [20]. Furthermore, Corolia and Noventi [21], saponin compounds can work as an antimicrobial. These compounds can damage the cytoplasmic membrane and kill cells.

Based on the research data, it is known that the hatchability of *B. gonionotus* fish eggs during the study was 39-88% (Table 1). The hatchability of eggs in this study was higher than that stated by Agustin and Rahardja [22], namely the hatchability of *B. gonionotus* fish generally only able to produce 10,000 eggs with a low hatchability of 22%. In this study, the concentration of *C. gigantea* leaf extract (800 ppm) resulted in the highest hatchability of fish eggs at 88%. This proves that the antifungal content contained in the leaves of *C. gigantea* with a concentration of 800 ppm is optimal in inhibiting the fungal infection of *Saprolegnia* sp. on the eggs of the *B. gonionotus* fish. Mulya's research [11] said that the use of *C. gigantea* leaf extract at a concentration of 1200 ppm was able to inhibit *Saprolegnia* sp. and increase hatchability of squid eggs with hatchability of 87.75%. However, in this study it was also known that the higher the concentration of *C. gigantea* leaf extract (above 800 ppm) could cause a decrease in the hatchability of fish eggs. It is suspected that the active compounds in *C. gigantea* leaves at high concentrations can interfere with the metabolism of *B. gonionotus* fish eggs, causing the eggs to not hatch. Ghofur [14] suggested that the use of extracts with too high a concentration can interfere with egg metabolism so that the eggs cannot hatch. Andriyanto *et al.* [23] stated that the success of eggs to hatch is influenced by several factors, including internal factors, namely the mechanical work of the larval activity itself and from the enzymatic work produced by the eggs.

| Treatments | Hatching Rate (%) | Survival Rate (%) |
|------------|------------------|------------------|
| Control (0 ppm) | 53 ± 4.36<sup>b</sup> | 86.84±26.4<sup>ab</sup> |
| A (400 ppm) | 70 ± 4.36<sup>c</sup> | 87.06±2.90<sup>ab</sup> |
| B (800 ppm) | 88 ± 5.3<sup>d</sup> | 92.54±3.34<sup>b</sup> |
| C (1200 ppm) | 81 ± 6.1<sup>ed</sup> | 89.20±7.46<sup>ab</sup> |
| D (1600 ppm) | 50 ± 4.0<sup>ab</sup> | 81.80±6.17<sup>ab</sup> |
| E (2000 ppm) | 39 ± 2.6<sup>a</sup> | 78.70±2.71<sup>a</sup> |

Note: writing different superscripts in the column indicates a significant difference between treatments (P<0.05).

The survival value of *B. gonionotus* larvae for 14 days of rearing showed that the use of *C. gigantea* leaf extract had a significant effect (P<0.05) on the survival rate. During maintenance, the larvae were fed at the age of 2 days in the form of phytoplankton, and at the age of 4 to 14 days were given live food in the form of artemia. During the study, the survival rate of *B. gonionotus* larvae was high above 70% (Table 1). It is indicated that the chemical content in the leaves of *C. gigantea* is also able to boost the immune system so that fish can survive from fungal pathogen attacks. Dalimartha [24] stated that the leaves of *C. gigantea* contain saponins, flavonoids, polyphenols, tannins, and calcium oxalate which can
be used as anti-microbial agents. The highest survival value of *B. gonionotus* was found in treatment B (800 ppm) which was 92.54% and the lowest was found in treatment E (2000 ppm) with a value of 78.70%. The survival rate for each treatment is quite good, where this figure is appropriate based on the Ministry of Agriculture [25] where the survival rate of good fish is on average 63.5% - 86%. Based on the results of observations of water quality parameters during the study on the rearing media of *B. gonionotus* fish larvae for 14 days of rearing, the value of water quality parameters still showed tolerance for growth and spawning processes (Tabel 2).

### Tabel 2 The range of the results of measuring water quality parameters for 14 days of maintenance

| Treatments | Temperature (°C) | pH | DO (mgL⁻¹) |
|------------|------------------|----|-------------|
| Control (0 ppm) | 26.6 – 26.7 | 6.8 – 6.9 | 5.7 – 5.7 |
| A (400 ppm) | 26.5 – 26.8 | 6.7 – 7.0 | 5.6 – 5.6 |
| B (800 ppm) | 26.5 – 26.8 | 6.7 – 6.9 | 5.4 – 5.7 |
| C (1200 ppm) | 26.5 – 26.9 | 6.9 – 6.8 | 5.3 – 5.6 |
| D (1600 ppm) | 26.5 – 26.8 | 6.8 – 6.8 | 5.4 – 5.7 |
| E (2000 ppm) | 26.5 – 26.9 | 6.8 – 6.8 | 5.4 – 5.7 |

Water quality parameters are important in the process of hatching fish eggs and the spread of fungi. Based on observations during the study, the temperature ranged from 26.5-26.7°C. Every organism has the ability to adapt to certain limits for its life. According to Santoso dan Wikatma [26] temperature for the habitat of *B. gonionotus* ranges from 20-33°C. The results of the measurement of the pH of show ranges from 6.7-7, the pH range is still within limits for growth and hatching of *B. gonionotus* fish eggs. Furthermore, the results of DO measurements show an average of 5.5-5.7 mgL⁻¹.

### 4. Conclusion

Based on the results on the study, it can be concluded that the use of *Calotropis gigantean leaves* extract significantly affected the hatchability eggs and survival of *Barbonymus gonionotus* larvae, where a concentration of 800 ppm produced the highest value for hatchability of fish eggs (88%) and survival rate (92.54%).

### Reference

[1] Diamsyah S, T Amarullah, S Rahmita, Sukardi 2018 *Jurnal Akuakultura* 2 33-39  
[2] Maulida S, K Eriani, F M Nur, N Fadli, A S Batubara, A A Muhammadar, M N Siti-Azizah, M Wilkes, Z A Muchlisin 2021 *Brazilian Journal Of Veterinary Research And Animal Science* 58 1-8  
[3] Alfath Z, F basuki, R A Nugroho 2020 *Sains Akuakultur Tropis : Indonesian Journal of Tropical Aquaculture* 4 129-138  
[4] Lingga M N, I Rustikawati, I D Buwono 2012 *Jurnal Perikanan dan Kelautan* 3 75-80  
[5] Rezinciuc S, Sandoval-Sierra J V, Diéguez-Uribeondo J 2014 *Journal Fungal Biology* 118 591-600  
[6] Magray A R, S A Lone, B A Ganai, F Ahmad, S Hafeez 2021 *Journal Aquaculture* 542 736876  
[7] Zuraidah S, Sillkhai 2016 *Jurnal Perikanan Tropis* 3 119-130  
[8] Rosidah, Y Andriani, W Lili, I Herdiawan 2017 *Jurnal Perikanan dan Kelautan* 7 199-209  
[9] Ghofur M, M Sugihartono, R Thomas 2014 *Jurnal Ilmiah Batanghari Jambi* 14 37-44  
[10] Seniya C, S S Trivedia, S K Verma 2011 *Journal of Chemical and Pharmaceutical Research* 3 330-336  
[11] Mulya A 2016 Efektifitas Ekstrak Metanol Daun Widuri (*Calotropis gigantea*) terhadap *Saprolegnia* sp. Pada Telur Ikan Peres (*Osteochilus kappenii*) Skripsi Jurusan Budidaya Perairan Unsiyah  
[12] Gavamukulya Y, F Abou-Elellya, F Wamunyokoli, H Ael-Shemy 2014 *Asian Pacific Journal of Tropical Medicine* 7 2355 - 5363
[13] Webster J, R Weber 2007. Introduction To Fungi 3rd Edition. Cambridge University Press. Singapore pp 1-817
[14] Ghofur M, M Sugihartono, R Thomas 2014 Jurnal Ilmiah Universitas Batanghari Jambi 14 37-44
[15] Arfiah H, Muftucha, O Carman 2006 Jurnal Akuakultur Indonesia 5 103-112
[16] Effendie M. I 1979 Metode Biologi Perikanan Yayasan Dewi Sri Bogor 112 hlm
[17] Sharma P, J D Sharma 1999 Journal of Ethanopharmacology 68 83 –95
[18] Mushir A, N Jahan, A Ahmeed 2016 Journal Discovery phytomedicine 3 15-21
[19] Ulina G V B, Sumardianto, Romadhon 2016 Journal Pengolahan dan Bioteknologi Hasil Perikanan 5 64-70
[20] Sulistyawati D, S Mulyati 2012 Jurnal Biomedika 2 47-51
[21] Carolia N, W Noventi 2016 Jurnal Majority 5 140-145
[22] Agustin F, B S Rahardja 2013 Jurnal of Aquaculture and Fish Health 2
[23] Andriyanto W, B Slamet, I M D J Ariawan 2013 Jurnal Ilmu dan Tekonologi Kelautan Tropis 5 192-207
[24] Dalimartha S 2003 Atlas Tumbuhan Obat Indonesia Jilid 3 Puspa Swara Jakarta 129 hlm
[25] Deptan 1997 SK. Mentan No. 940/Kpts/OT.210/10/1997 Pedoman Kemitraan Usaha Pertanian. http://deptan.go.id Jakarta Departemen Pertanian. Diakses pada tanggal 4 Januari 2019
[26] Santoso B, T S Wikatma 2001 Petunjuk Praktis Budidaya Tawes (Yogyakarta: Penerbit Kanisius) 44 hlm