The effect of frangipani leaf extract (*Plumeria* sp.) on the hatchabiiity and growth of sangkuriang catfish (*Clarias gariepinus*) larvae

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Abstract. Catfish (*Clarias gariepinus*) or in local name *Lele sangkuriang* is a type of freshwater fish that is often cultivated in the worldwide community particularly Indonesia. The market demand for this fish is increasing every year so that fish farmer can get better profit in the future. In order to prevent a decrease in seed production in the future, the seed procurement process must be carried out. Moreover, to ensure the production levels must be continued to increase both in quantity and quality. This research aimed to determine the effect of frangipani leaf extract (*Plumeria* sp.) on hatchability rate and survival of the larvae of catfish (*Clarias gariepinus*). The experimental method in this research was completely randomized design with 5 treatments and 3 replications. The treatments used were P0 without frangipani extract (control), P1 giving frangipani extract 20 ppm, P2 giving frangipani extract 40 ppm, P3 giving frangipani extract 40 ppm and P4 giving frangipani extract 80 ppm. The results showed that different doses of frangipani leaf extract produced different effects on hatchability and the quality of the larvae produced. The highest percentage of hatchability results was obtained in treatment P1 (85.33%) and the best percentage of larvae survival rate was in treatment P2 (72.67%).

1 introduction

Public demand for the consumption of catfish from aquaculture is increasing every year [1]. In order to fulfil Indonesian domestic demand, an increase in the production of catfish should be carried out every year [2]. The total production of cultured catfish in 2020 was 347.511.48 tons, this production capacity tends to decrease compared to previous years, namely in 2018 it produced 1.027.032,54 tons and in 2019 as much as 981.623,40 tons [3]. Catfish (*Clarias gariepinus*) or *lele sangkuriang* in local name is a popular fishery commodity among the public [4, 5, 6]. This fish originated from Africa and was imported to Indonesia in 1984. Catfish is one type of fish that is most easily accepted by the public because of its advantages [7]. The nutritional composition of catfish is protein (17.7%), fat (4.8%), mineral (1.2%), and water (76%) [8]. Catfish have advantages compared to other types of fish, namely having faster growth, having high tolerance for poor water quality, and being more resistant to disease attacks and can be kept in all cultivation containers [9].

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However, there are many problems that arise such as disease attacks in the egg and larval phases [10]. One of disease that is often and commonly found is Saprolegniasis caused by the *Saprolegnia* fungus. It is often found that the Saprolegnia fungus infects catfish in the age phase to the adult phase, *Saprolegnia* is a disease that is often found attacking fish in fresh waters [11]. In the intensive hatchery stage, problems are often found, namely disease attacks by *Achyia* fungi and *Saprolegnia* sp. which cause a decrease in the hatching rate of the eggs, and during the incubation period many dead fish eggs are found due to fungi [12].

The use of chemical drugs has a negative impact on fish life, namely the killing of non-target organisms, the emergence of immunity to pathogens, affected growth and reproduction and causing pollution. So that a safe alternative is to utilize herbs that are more environmentally friendly and easily decomposed by water, and also herbs have relatively small side effects [13, 14]. The use of herbs as a therapy for fish diseases has been carried out in previous research. Uses of *Lonicera japonica* Thunb. components can increase the resistance of grouper fish in vitro and in vivo [14]. Then extract *Illicium verum* Hook. f. has been proven to be able to resist grouper virus attacks [14]. *Curcuma kwangsiensis* ingredients have also shown significant results against grouper iridoviral infection in vitro and in vivo [15]. Apart from that, several marine based plants and their active ingredients have also been proven to increase the immunity of *Litopenaeus vanname* shrimp against disease attacks such as *Sargassum oligocystum*, carrageenan, *Spirulina* sp. and fucoidan [16, 17, 18, 19]. The use of *Plumeria* sp. as a therapeutic treatment and to help in the hatching and larval capacity of catfish has never been done before. In this study, we used Plumeria species that contain tannins, flavonoids, steroids, saponins, terpenoids that show antibacterial activity [20]. Therefore, the aim of this current study is to examine the effect of different concentrations of frangipani leaf extract *frangipani* leaf extract on the hatching rate and survival rate of catfish, *Clarias gariepinus*.

2 Materials And Methods

2.1 Experimental Design

This research was conducted in December 2022. Located in the laboratory of the Faculty of Marine and Fisheries of Syiah Kuala University, Darussalam, Banda Aceh, Aceh. The tools and materials used in this research were pH meter, thermometer, DO meter, aerator, refractor, evaporator, blender, NaCl, chicken, digital camera, basin, plastic cup, 70% ethanol, shaker, pH meter, scales, ruler, frangipani leaf extract. The experimental design used was a complete randomized design (CRD) using 5 treatments and 3 replications. The treatments are as follows:

P0 = without using frangipani leaf extract (*Plumeria* sp)
P1 = using 20 ppm frangipani leaf extract (*Plumeria* sp.)
P2 = using 40 ppm frangipani leaf extract (*Plumeria* sp.)
P3 = using 60 ppm frangipani leaf extract (*Plumeria* sp.)
P4 = using 80 ppm frangipani leaf extract (*Plumeria* sp.)

2.2 Research Procedure

A 15 units aquarium sized 80 x 40 cm were used during the study. Catfish eggs were stocked as many as 100 fish/container. The fish eggs were first soaked in a geyser for 1 minute with a solution of frangipani leaf extract and placed on pet topes. For the extraction process, frangipani leaves were washed thoroughly so that impurities such as dust that stick to the frangipani leaves are removed. Next, the frangipani leaves were dried without exposure to sunlight. The dried frangipani leaf samples were made into powder by pulverizing. Extraction

was carried out using maceration method with 70% ethanol solvent as modified method by previous work [21]. A total of approximately 800 g of frangipani leaves were soaked with 1,600 mL of 70% ethanol for 3 days, covered, stored in a dark room and then shaken with a 120 rpm mixer for 7 days. Afterward, the filtrate was removed and the residue was re-macerated with 50 mL of 70% ethanol. Subsequently, the filtrate was removed every day for 7 days. After that, maceration ended. After the extract filtrate from frangipani leaves is obtained, it was concentrated by using a rotary-evaporator at a temperature of ± 40ºC, until a ready-to-use extract is obtained.

3 Research Parameters

3.1 Hatching Rate (HR)

To measure hatching eggs divided by the number of fertilized eggs multiplied by one hundred percent and expressed in (%). According to [22] the degree of hatching of eggs can be calculated using the formula:

$$HR = \frac{number\ of\ hatches}{number\ of\ fertilized\ eggs} x100$$

3.2 Survival Rate

SR calculation was done at the end of the study after 35 days. According to [23], the survival rate is expressed in terms of the percentage of living fish at the beginning and end of the study and is formulated as:

$$SR = \frac{Nt}{N0} 100\%$$

Description:
SR = Survival Rate (%)
Nt = Number of fish at the end of the study (ind)
N0 = Number of fish at the begin of the study (ind)

3.3 Length Gain

Length gain is defined as the total gain in length and final weight minus the length of begin weight. Mutual length gain was calculated based on the formula used by [24] :

$$Lm = Lt - Lo$$

Description :
Lm = Length gain (cm)
Lt = Average length of fish at the end of the study (cm)
Lo = Average length of fish at the beginning of the study (cm)

3.4 Abnormality

Abnormality in the larval phase is in the form of bending of the body and tail [25]. Observations of abnormality in this study include head shape, body shape and tail shape. Calculation of abnormality can be done using the formula [26], which is as follows:

$$Abnormality = \frac{number\ of\ abnormal\ larvae}{number\ of\ normal\ larvae} x100$$

3.5 Data Analysis

All data in this study were analyzed using analysis of variance (ANOVA) in accordance with a complete randomized design (CRD). The average treatment will then be further tested using the Duncan test with a determination of $p < 0.05$ to determine the effect of differences between treatments.

4 Results And Discussion

Result showed that the treatment frangipani leaf extract immersion has a very significant effect on the hatching rate of catfish eggs. This is assumed due to frangipani leaf extract has antifungal properties. According to [27] frangipani leaves showed positively contain flavonoids, alkaloid and saponins. Flavonoid compounds have activity as anti-bacteria with the mechanism of inhibiting the work of nucleic acid synthesis, inhibiting the function of cell membranes, and inhibiting energy metabolism. The lowest value was obtained in the P4 treatment (80 ppm) with value 34.67%. According to [28] because the higher the concentration of the extract, the greater the risk of egg damage. Excessive levels of antioxidants cause toxicity and mortality of fish eggs. In the treatment of P0 (control) has a low value of 15.67%. This is due to the growth of mold that causes further damage to healthy eggs, causing eggs to die and not hatch. According to [29]eggs attacked by mold will show signs of fine threads, such as cotton and white paint, formed around the egg.

Previous results reported by [30] on the effect of soaking doses of *Syzygium aromaticum* leaf extract on the hatchability of catfish, *Clarias gariepinus*, where the use of clove leaf solution at certain concentrations has a significant effect ($P > 0.05$) on hatchability. The similar result were in line with [13] studied the effect of using *Avicennia marina* leaf extract on the hatchability of *Clarias gariepinus*. *Avicennia marina* leaf extract immersion proved to have a significant effect on hatchability of catfish ($P<0.05$). The use of Moringa leaves (*Moringa olifera* I.) at different concentrations has also significant effect on the infection rate and hatchability of *Clarias gariepinus* [31]. The use of kirinyuh leaf extract (*Euphatorium odoratum* l.) had significant effect on the hatching rate of milkfish *Chanos chanos* [21]. Moreover, egg hatchability and survival larvae of Pomfret Fish (*Colossoma Macropomum*) has also stimulated by turmeric extract (*Curcuma Domestical*) [32]. Therefore, based on previous research, it is suspected that the addition of frangipani leaf extract with a certain concentration has a significant effect on hatching fish eggs as was done in this research.

Table 1. Hatchability, survival, length gain, and abnormality results

Frangipani leaf extract treatment					
Parameter	P0 (0 ppm)	P1 (20 ppm)	P2 (40 ppm)	P3 (60 ppm)	P4 (80 ppm)
HR (%)	15.67±9.59^{a}	85.33±10.06^{de}	80.00±10.06^{d}	53.33±10.01^{c}	34.67±9.89^{b}
SR (%)	13.67±5.61^{a}	71.33±5.88^{d}	72.67±5.88^{de}	47.33±5.85^{c}	29.33±5.78^{b}
Length gain (cm)	0.77±0.04^{c}	0.72±0.04^{a}	0.72±0.04^{a}	0.75±0.04^{bc}	0.73±0.04^{ab}
Abnomality (%)	0	0	0	1±1.50^{d}	3.67±1.50^{e}

Table 1 showed that the highest larva survival was found in the P2 treatment (40 ppm) with a value of 72.67%, this value is not much different from the P1 treatment (20 ppm) with a value of 71.33% and the lowest average in the P4 treatment (80 ppm dose) with a value of 29.33%. In this study, the survival of larvae was influenced by the hatchability of the eggs

themselves, and apart from that the mortality of larvae according to [33] is usually due to the age and size of the fish able to survive. According to [34] stated that environmental factors such as handling and water quality affect the survival rate of fish. In this study, water quality is always maintained by drinking regularly to remove feces and uneaten fish feed residues that affect water quality. This research has results that are in line with the research of [35], namely feeding the type of beut fish extract provides a very significant difference in survival (SR) of catfish larvae and research from [36] on the effectiveness test of papaya leaves (*Carica papaya*) to treat *Aeromonas hydrophila* bacterial infection in carp (*Carassius auratus*). Then also in line with [37] on soaking catfish (*Clarias* sp.) in guava fruit juice for the treatment of *Aeromonas hydrophila* infection. Another herb like ginger (*Zingiber officinale*) can enhance the immunity of Tilapia (*Oreochromis niloticus*) [38]. But different result were reported by [39] on the effect of kersen leaf extract (*Muntingia calabura*) with different doses on incubation time, hatchability and larval life of catfish (*Clarias gariepinus*). Based on previous research, it is suspected that the addition of frangipani leaf extract with a several concentration active compound has a significant effect on the survival of fish larvae.

It can be seen that the highest growth (length gain) was obtained in P3 treatment or dosing of 60 ppm extract with a value of 29.33 cm and the lowest average was obtained in P1 and P2 or dosing of 20 ppm and 40 ppm extracts with an average value of 0.72 cm. All larvae in this study had similar growth. Larvae development under captive condition are influenced by several factors like nutrition, environment and rearing technique [40]. According to [41] expressed that the balanced nutritional content (protein, fat and fiber) in fish feed accelerates relatively faster fish growth. The use of supplementation has also significant effect in supporting fish (including shrimp) growth [7, 42, 43]. Previous result has reported the administration of probiotic could boost the significant growth in aquaculture [44, 45, 46, 47]. However some supplementation could also had negative impact on fish or shrimp culture [48].

The results showed that the highest percentage of abnormality rate with a value of 3.67% was obtained from the P4 treatment followed by P3 with a size of 1%, while for treatments P0, P1, and P2 no abnormality larvae were found. The success of a hatchery is not only seen from its egg-laying capacity, but also from the quality of the resulting larvae, such as the level of abnormality of the larvae. This research can be considered to have a better percentage than the research of [49] on the effect of soaking clove leaf extract (*Syzygium aromaticum*) on the hatchability of peres fish (*Osteochillus kappeni*) and the research of [50] the utilization of pineapple leaf extract in determining the survival rate of milkfish larvae. But it is not the same as the research of [12] on the effectiveness of kecombrang flower extract (*Nicolaia speciosa horan*) for the prevention of *Saprolegnia* sp. attack on catfish which did not have any abnormality in its research.

Table 2. Water Quality Parameters

	Water Quality Parameters		
Treatment	**DO (ppm)**	**Temperature ºC)**	**pH**
P0	4-7	27-31	6.8-7.2
P1	4-7	27-31	6.8-7.2
P2	4-7	27-31	6.8-7.2
P3	4-7	27-31	7-7.2
P4	4-7	27-31	7-7.2
Reference [9]		25-32	6-8.5

Table 2 showed the water quality parameters during the study. The dissolved oxygen (DO) values obtained ranged from 4-7 ppm. The dissolved oxygen content that can be tolerated by catfish in aquaculture is a minimum of 3 mg/L [6]. The temperature values obtained during the research ranged from 27-31ºC. This is in line with the research of [51] on different feeding

on the growth and survival of catfish larvae *Clarias gariepinus* can survive at temperature range of 27-30ºC. The measurement of the pH value of the water during the study was 6.8-7.2 which is also in line with report from [52]. Thus it can be concluded that the water quality parameters in this research have been well organized and optimal.

5 Conclusion

The administration of frangipani leaf extract has significant effect on the hatchability of catfish *Clarias gariepinus.* Moreover, high dose of frangipani leaves inhibits the hatchability of the eggs themselves due to the concentrated antioxidant content. Frangipani leaf extract affects the survival of the resulting larvae, where at appropriate doses a high survival rate is obtained, but compared to high doses the resulting larvae have abnormalities that affect their development.

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