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EGGS EFFECTIVENESS LEAVES EXTRACT *PIPER BETLE* TO ATTACK PREVENTION FUNGUS *SAPROLIGNIA SP* IN *OSTEOCHILUSHASSELTI*

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ABSTRACT

Betle leaf (*Piper betle*) is one of the traditional native plant to Indonesia that known to contain antifungal substances. The betle leaves contained essential oils, tannins, phenoids, saponnis that able to inhibit the growth of fungi. This study aims to determine the optimal extract concentration of betle leaf on egg hatchability and survival of nilem larvae. This study used an experimental method with a completely randomized design consisting of 5 preparations and 3 replications. The treatment given is soaking nilem fish eggs for 20 minutes into the media with betle leaf extract contents namely P1: 0 ppm (control), P2: 20 ppm, P3: 40 ppm, P4: 60 ppm, and P5: 80 ppm. Test parameters that observed including egg hatchability, larval survival, prevalence of fungal infection and water quality. The results of egg immersion in betle leaf extract at a concentration of 80 ppm (P4) showed the best results with 95 ±3,36% hetching rate off eggs. The prevalence of fungal infection on P5 was 3.67 ± 2.46%, and the larval spreading on P4 was 87.34 ± 3.17%. Water quality in the study showed a pH value of 7, temperatures between 21 - 23^{o}C, and dissolved oxygen of 6.06 -7 mg / L.

KEYWORDS: *Osteochilus hasselti egg, Betle Leaf Extract, prevalence fungus Hatching Rate, Intensity of Fungal Infection.*

INTRODUCTION

Nilem fish is an endemic fish of Indonesia that normally lives in fresh water with clear water quality and is one of the freshwater aquaculture commodities concentrated on the island of Java. Nilem fish (*O. hasselti*) is a freshwater fish that is not only popular with meat but the eggs are also consumed by the people. Nilem fish eggs have a delicious flavor and can be explored in certain countries. High public interest in nilem makes nilem fish production continue to increase (Firdaus *et al*., 2017). This causes the nilem fish must be cultivated to meet the demand so that fish stocks are maintained.

One obstacle in the cultivation of nilem is the availability of seeds that are not continuous. The availability of seeds is determined by the number of eggs that hatch.

The hatching of eggs is the process of developing an embryo in the egg until it hatches (Sa'diah et al., 2015). At the egg hatching stage, nilem fish often experience problems, one of which is the egg does not hatch. Eggs that do not hatch are usually caused by fungus. Eggs that are attacked by fungi can cause eggs to not hatch or rot so that the hatching rate is low (Dian *et al*., 2015). Overcoming this, prevention is needed.

One of the efforts made in preventing eggs attacked by fungi is usually by adding chemicals whose purpose is to inhibit the growth of fungi in eggs. Chemicals that are frequently used include Methylene blue, formalin, melatchine green, NaCl, KmNO4, (Rivanto *et al*., 2014). The use of synthetic chemicals including antibiotics can have a resistant effect on pathogenic microbes and cause environmental pollution if their use is inappropriate (Sabrina *et al*., 2014).

Overcoming this problem, it is necessary to have an alternative prevention by using materials that are environmentally friendly and do not cause effects resistant to fungi and certainly can prevent fungal diseases (Rosidah *et al*., 2017). One alternative is to use traditional plants that are anti-fungal. In addition to being antifungal, these plants are also easily obtained and are easy to use for preventing and treating fish or egg disease(Zuraidah and Silkhairi, 2016). One of the traditional plants that can potentially prevent fungal diseases is the betel leaf plant (*Piper betle* L) (Rivanto *et al*., 2014).

MATERIALS AND METHODS

3.1.3. Material

The materials used for making the extract were fresh betel leaf, distilled water and filter paper. Other materials used for broodstock injection of nilem fish include ovaprim and aquadest.

3.1.Research methods

The research method used in this study is an experimental method using a completely randomized design (CRD) with 5 treatments and 3 replications so that it becomes 15 experimental units. The treatment given was soaking nilem fish eggs into media with different content of betel leaf extract, namely:

P1: Betel leaf extract concentration 0 ppm (control)

P2: Betel leaf extract concentration 20 ppm

P3: Betel leaf extract concentration 40 ppm

P4: Betel leaf extract concentration 60 ppm

P5: Concentration of betel leaf extract 80 ppm

The research variables observed were egg hatchability, fungal attack rate, and larval survival. The main data calculated were the number of eggs that hatched and the survival of larvae that were reared until the yolks were exhausted. Supporting data in theform of the number of eggs attacked by fungi and water quality include temperature, pH,and dissolved oxygen.

3.2. RESEARCH PROCEDURE

3.3.1. Production of betel leaf extract (*Piper betle* L)

Making betel leaf extract is done by selecting fresh betel leaves, then washed and drained without the help of sunlight. Then the betel leaves were dried using an oven at 60^{o}C for 1 day. Then the dried betel leaves are mashed using a blender which is then sieved with a sieve to obtain powder. Then the powder was dissolved using 1 liter of distilled water with the amount of simplicia according to the treatment (20 ppm, 40 ppm, 60 ppm, and 80 ppm). The powder was brewed with distilled water at 90^{o}C for 30 minutes. Betel leafextract can be used after cooling (Dwiyanti, 1996 in Zuraidah and Silkhairi, 2016).

3.3.2. Container Preparation

Container preparation includes soaking and maintenance containers. The container is a basin measuring 6 liters of water as many as 15 pieces. Previously, the basin was washedand dried,

then filled with water and given a solution of Potassium Permanganate for 24 hours. After that, it was rinsed and dried, then filled with 2 liters of water again with a water level of 4.8 cm and aerated for 24 hours.

3.3.3. Nilem Fish Spawning

Nilem fish spawning is done artificially. There are 2 brooders (1 female and 1 male) and the gonads are ripe and ready to be spawned. Before spawning, the female and male Nilem broodstock were weighed to determine the dose of ovaprim. The female nilem broodstockwere injected with 0.5 ml/kg ovaprim and the male parent was injected with 0.3 ml/kg ovaprim. After being injected, the broodstock is put into a fiber bath for the spawning process. After spawning, nilem fish eggs are ready to use.

3.3.4. Giving Betel Leaf Extract (Piper betle L)

The betel leaf extract was given using the soaking method for 20 minutes. Soaking was carried out in a 6 l basin with a volume of 1 l of betel leaf extract. 100 eggs were taken using a sieve, then soaked in betel leaf extract according to treatment for 20 minutes. After soaking, the eggs are transferred to a pre-prepared rearing basin. The immersion method refers to the research of Ghofur et al., (2014).

3.3.5. Maintenance of Nilem Fish Eggs

Eggs that have been transferred to the rearing basin are maintained until the eggs hatch. During the hatching process, each rearing container is aerated. After the eggs hatch, the eggs that hatch are counted and observed the eggs that are attacked by fungus by looking at the number of dead fish eggs with fungus and dead eggs without fungus. Eggs that areattacked by fungus are characterized by the presence of threads that cover the eggs like cotton. Then after hatching, the larvae are reared for as long as the egg yolk runs out. Then calculated to determine the survival value of nilem fish larvae.

RESULTS AND DISCUSSION

Research on the effect of betel leaf extract (Piper betle L) on egg hatchability and larval survival of nilem fish (Osteochilus hasselti) with 5 different treatments. The treatments were P1 (control), P2 (20 ppm betel leaf extract), P3 (40 ppm betel leaf extract), P4 (60 ppm betel leaf extract), P5 (80 ppm betel leaf extract).

4.1. Egg Hatchability

Observation of egg hatchability was carried out after ± 24 - 48 hours by counting the number of eggs that hatched into larvae (Marthin et al., 2018). Egg hatchability was obtained by observing fertilized eggs and hatched into larvae. The results of observationsof the percentage of egg hatchability by immersion treatment on betel leaf extract with different concentrations can be seen in Figure 1.

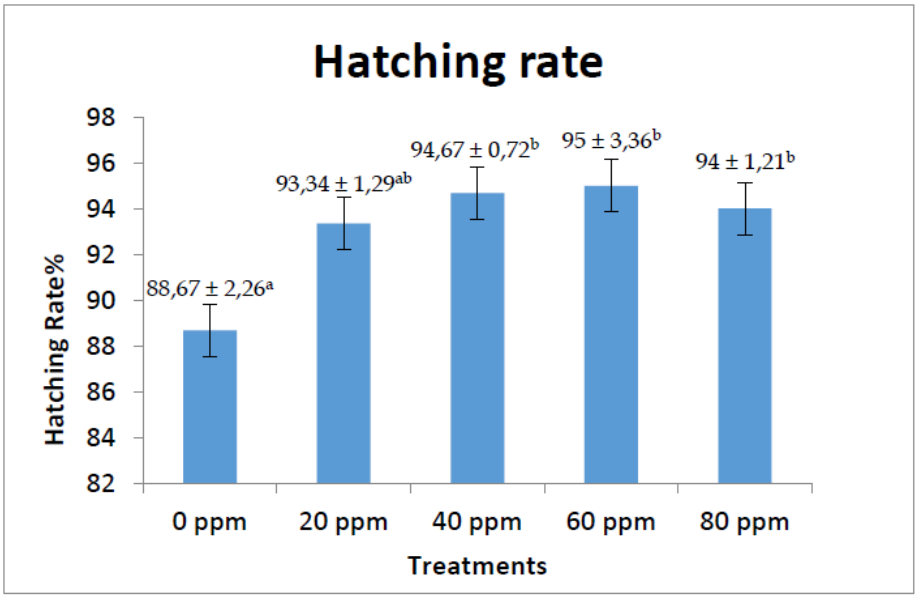

Figure 1: Hatchability of Nilem Fish Eggs.

Note: The same superscript letter indicates that the betel leaf extract with different concentrations has a significantly different effect (P<0.05).

The results showed an increase in egg hatchability at P1 (88.67 ± 2.26), P2 (93.34 ± 1.29), P3 (94.67 ± 0.72), P4 (95 ± 3.36), and P5 (94 ± 1.21). The results of statistical analysis (ANOVA) showed that the administration of betel leaf extract with different concentrations in each treatment showed a significant difference (P <0.05). Thestatistical test was continued with the Tukey Test. Tukey's test results showed that P1 was not different from P2 but significantly different from P3, P4, and P5. However, at P2, P3, P4, and P5 were not significantly different. The hatchability of eggs at P4 was the best to prevent the growth of fungus on the eggs. P4 with a value of 95 ± 3.36% showed the bestresults compared to P2, P3, and P5, presumably because the betel leaf extract contains various anti-bacterial or fungal compounds and the concentrations used are effective so that they are not harmful to egg development. Munurut Huzni et al., (2015), active compounds found in betel leaf such as alkaloids, flavonoids, and saponins are able to protect the chorion from fungal attacks.

In P3 the hatchability value of eggs was lower than in P4, this was presumably because the concentration of the extract given did not have an effect on resisting fungal attacks sothat the eggs would also rely on chorion to resist fungal attacks. According to Sumantadinata (2012), if the embryonic development process is attacked by fungi, the ability of the eggs to hatch will be reduced and even cause the death of the eggs so that the success of the eggs is low.

At P5 with a concentration of 80 ppm it was lower than P4 with a value of 94 ± 1.21%, this was caused by active compounds such as saponins which were able to poison organisms at certain concentrations. Inaya et al., (2015) confirmed that saponin compounds can inhibit egg development by damaging cells so that there is a change in the structure of the egg which causes fluid in the cell to come out and cell dehydration occurs. Cell dehydration that occurs will cause the eggs to fail to hatch, because in their development the eggs require fluids that contain nutrients.

4.2.The prevalence of fungal infection rate

Fish eggs are known to be relatively susceptible to fungal attack. The fungus will attack infertile (dead) eggs and attack healthy eggs. How to calculate the number of eggs infected with fungus according to Helmiati (2005) is the number of eggs infected with fungi divided by the number of initial eggs. Percentage of fungal attack rate obtainedduring the study:

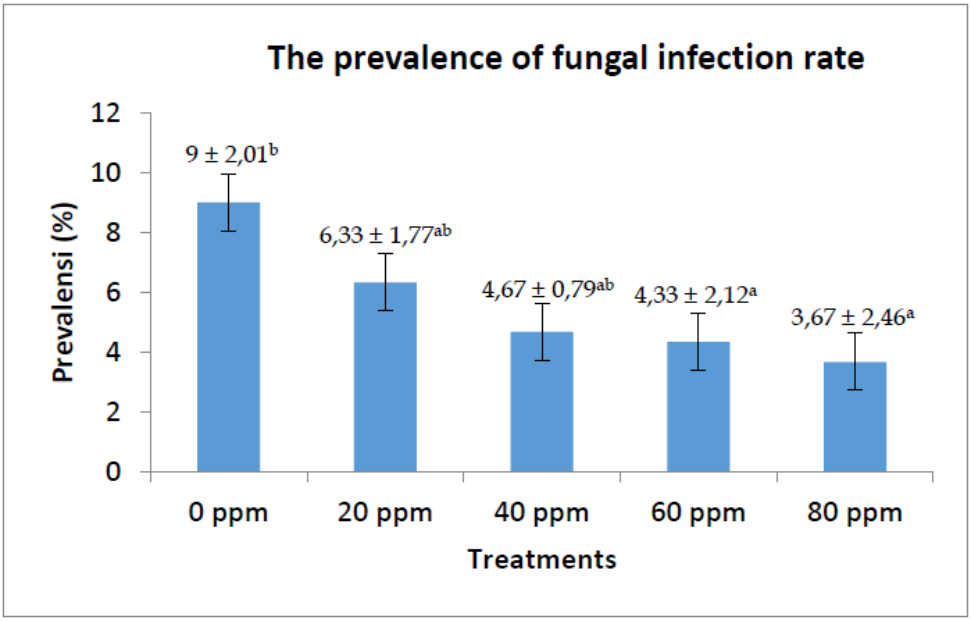

Figure 2: The prevalence of fungal infection rate.

Note: The same superscript letter indicates that the betel leaf extract with different concentrations has a significantly different effect (P<0.05).

Based on the results of the research that has been done, the more concentration given, theless the level of fungal attack. The results of the ANOVA test showed a significant difference ($P<0.05$) to the treatment. Tukey's test results show that P1 is not different from P2 and P3, but different from P4 and P5. At P1, P2, and P3 are not different, but different from P4 and P5. The results of the Tukey test showed that the lowest level of fungal attack was found in P5 with a value of 3.67 ± 2.46%, it is suspected that the concentration given to P5 was more capable of inhibiting fungal growth. Rivanto et al., (2014), added that the higher the concentration of betel leaf extract to a certain extent willbe able to kill the fungus but also the high concentration of the extract will be absorbed by the eggs so that it is toxic to the eggs and causes the eggs not to hatched.

Eggs that were not soaked with betel leaf extract in P1 (control) gave the fungus Saprolegnia sp. the highest is 9 ± 2.01%. The high fungal attack is because the eggs are not soaked by betel leaf extract so they are not protected by antifungal substances contained in the extract, so the fungus will easily stick to the eggs, enter and infect the eggs. This causes uncontrolled fungal growth so that it continues to attack healthy eggs and results in healthy eggs that die and do not hatch. According to Effendie (2001), fungalattacks can weaken the chorion, then it becomes wrinkled because the fungus attaches and penetrates the chorion to take the food substances in it.

According to Fitri (2007), eggs are a good growing medium for microorganisms, becauseeggs contain chemical compounds that serve as a source of nutrition for these microorganisms. Without antifungal compounds, the resistance of eggs to attack by the fungus Saprolegnia sp. weakened and only rely on the strength of the chorion alone. Eggsattacked by Saprolegnia sp. Visible hyphae in the form of fine threads attached to the surface of the egg. The eggs that do not hatch due to the attack of the fungus Saprolegniasp. appears to show signs around the egg there are fine threads like white cotton. This is in accordance with the opinion of Kordi (2004), who said that eggs that are attacked by fungi will usually appear covered with thread-like formations known as white fungal hyphae.

Unhatched eggs are thought to be the influence of low water quality Eggs thatdied were not attacked by fungi did not show the presence of hyphae of Saprolegnia sp.on the surface of the fish eggs. According to Rosidah et al., (2017), the greater the concentration given, the higher the average percentage of dead eggs.

4.4. Water quality

The results of water quality measurements during the study took place the temperature values in the morning ranged from 22 - 23 oC and in the afternoon ranged from 22 - 24 oC. From the results of temperature measurements during the study, it can be said that it is still in the normal range. Where according to Sunarma (2004), the normal temperature for hatching eggs is 22-32 C. While Saprolegnia sp. can grow at a temperature of 0-35 C with the best growth temperature in the range of 15-30 C and pH 4-6 (Irianto, 2005). According to Rozaldi, et al., (1990), stating that the speed of mushroom growth is closely related to environmental temperature, in general Saprolegnia mushrooms can grow at a minimum temperature of 0-5 C and an optimum of 15-30 C. So eggs that do not hatch are not caused by poor water quality but by Saprolegnia sp. or treatment.

The results of measuring the pH of the water during the study were 7. Based on the PP No. 82 of 2001 (class II), a good pH for aquaculture activities is in the range of 6-9. According to Amalia and Subanditono, (2013) that acidity (pH) that is not optimal can cause fish stress, disease susceptibility, productivity, and low growth. A low pH can cause a decrease in growth rate, and a high pH will increase ammonia which is indirectly harmful (Paulinus and Revol, 2015). So from the results of the pH measurements above, it shows that the pH during the study was within the feasibility range. Dissolved oxygen levels during the hatching process averaged 6.44 mg/L, while at the time of rearing nilem fish larvae, dissolved oxygen averaged 6.73 mg/L. Thus, the dissolved oxygen level during the study was still within reasonable limits for hatching Nilem fish eggs. Sumarna (2004) confirmed that a good range of dissolved oxygen is > 3 mg/L. From the results of this study, the dissolved oxygen content is still within the tolerance limit. The provision of dissolved oxygen in the hatching media water is needed as an oxygen supply through the addition of aeration. The oxygen enters the egg by diffusion through the surface layer of the egg shell. If oxygen levels are low, it will affect biological functions and can even lead to death (Sutisna and Sutarmanto, 1995).

CONCLUSIONS

Based on the results of the study, the following conclusions can be drawn:

1. Soaking eggs with different concentrations of betel leaf extract has an effect on egg hatchability and survival of nilem fish larvae.
2. The best concentration was at P4 with egg hatchability value of 95 ± 3.36%.

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