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Effectiveness of Bitter Melon (*Momordica charantia*) Solution for Masculinization of Siamese Fighting Fish (*Betta splendens*) Through Larval Immersion Method

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ABSTRACT

Siamese fighting fish (*Betta splendens*) is one of the fisheries commodities in great demand because it has an attractive physique and relatively high economic value. The increasing market demand for male Betta means a method is needed to produce male offspring en masse. This research aimed to determine the effect of bitter melon (*Momordica charantia*) solution on the male sex ratio of Betta by immersion of larvae in different concentrations. This research used an experimental method with a completely randomized design (CRD), which included four treatments with three replications, namely control treatment, P2 treatment with a concentration of 1.5 ml/l, P3 treatment with a concentration of 3 ml/l, and P4 treatment with a concentration of 6 ml/l. The data obtained was analyzed using the ANOVA method. If the data analysis showed a significant effect, it was carried out using Duncan's advanced test. The research showed that the treatment applied had a real influence on the percentage of male Betta but did not significantly affect the survival rate. The highest sex percentage value for Betta was found in treatment P4 (6 ml/l), which was 77%.

INTRODUCTION

Siamese fighting fish (*Betta splendens*) is an ornamental fishery commodity in great demand by the public. Siamese fighting fish or Betta have quite a high potential for cultivation; apart from being economical, they also have a unique and attractive color and body shape, so they are in demand by ornamental fish fans, especially male Betta. According to Dwinanti *et al*. (2018), the body shape of male Betta is slimmer; the color is brighter, and the fins are more extended and shaped like a fan compared to female Betta. Apart from that, Betta's price is relatively high. The increasing demand for male Betta means a method is needed to produce male offspring en masse. Nazar *et al.* (2017) stated that the solution offered to increase the male betta population could be masculinization by directing female genitals to males.

Masculinization in Betta usually uses the hormone 17 α-methyltestosterone. This synthetic hormone is very dangerous, especially for the animals being tested; it can cause death and can cause waters to become polluted (Rahmasari *et al*., 2021). The use of synthetic materials needs to be replaced with other alternative materials, such as the use of materials

containing steroid hormones, which are safe, cheap, and easy to use, especially for masculinization activities. The ingredients are sourced from natural ingredients containing stigmasterol, an aphrodisiac. The Lunasia amara plant plays a role in increasing the libido of male eastern deer because it is an aphrodisiac that has the potential to be applied to fish. The celery plant is an aphrodisiac because it contains flavonoids, saponins, and steroids (Awaludin *et al*., 2019). Apart from these natural ingredients, what is considered an aphrodisiac is bitter melon fruit (*Momordica charantia*).

Bitter melon is a natural ingredient that can substitute synthetic hormones, which play a role in masculinizing Betta. Prakoso *et al*. (2016) state that bitter melon contains active ingredients: flavonoids, glycosides, triterpenoids, steroids, saponins, and alkaloids. Awaludin *et al*. (2019) stated that masculinizing fish using purwoceng (*Pimpinella pruatjan*) extract with a concentration of 20 mg/L for 8 hours when immersion the larvae produced 73.3% male tilapia fish. Apart from purwoceng, ginseng root extract produces 95.05% with a concentration of 3 mg/L for 8 hours, and another ingredient that can be used for masculinization, namely celery extract, produces a percentage of male Siamese fighting fish of 73.33% with a concentration of 10 mg/L for 8 hours (Ferdian *et al*., 2017). Based on this, the researchers intend to use bitter melon, which contains steroid compounds, to direct the sex of Betta to become male. This research aimed to determine the effect of bitter melon (*Momordica charantia*) solution on the male sex ratio of Siamese fighting fish (*Betta splendens*) by immersion in the larvae.

METHODS

This research was carried out from 12 May to 23 July 2022 at the Fish Production and Reproduction Laboratory, Department of Fisheries and Marine Sciences, Faculty of Agriculture, Mataram University. The tools and materials used in this research were a pH meter, dissolved oxygen meter, thermometer, jar, aerator, camera, container, book, injection, scoop, filter, plastic spoon, plastic bottle, Betta larvae, bitter melon solution, artemia, salt, methylene blue, and ketapang (*Terminalia catappa*) leaves.

This research used an experimental method with a completely randomized design (CRD) including 4 treatments and 3 replications, namely Treatment P1 without immersion in bitter melon solution, Treatment P2 with a concentration of 1.5 ml/l, Treatment P3 with a concentration of 3 ml/l and Treatment P4 with a concentration of 6 ml/l. The container used is a 5-l jar for immersion in the larvae and spawning brood, while it uses a 10-l jar for rearing. Preparation is done by filling 15 cm of water for the spawning container, 2-l for immersion, and 5-l for rearing Betta. The water comes from wells from the Aquaculture Study Program at Mataram University.

The containers prepared for spawning are 3 jars and 12 jars for immersion and maintenance. The spawning process lasts 7 days until the fry is found and then reared until they reach 5 days. Betta larvae that have reached the age of 5 days are treated by immersion in bitter melon solution for 8 hours with the specified treatment group. Each jar is filled with 20 Betta larvae. Before the Betta larvae are put into the immersion container, acclimatization is first so that the larvae are not stressed due to drastic temperature changes. After immersion, the larvae are transferred to the rearing container. The larvae are kept for 60 days until physical characteristics are visible to differentiate the sex of male and female Betta. Maintenance is done by feeding them in the morning and evening, giving them artemia after the 1.5-month-old larvae are fed silkworms. Providing ketapang leaves and salt in the rearing

media aims to maintain Betta's health. Control during the research was carried out by changing the media water once every 5 days, 20-30% of the total amount of water.

Research parameters

Percentage of Male Betta

According to Indreswari *et al*. (2017), the percentage of male Betta is calculated using the following formula:

$$M = \frac{Number\ of\ male\ fish}{Number\ of\ samples}\ x\ 100\% \quad \text{..... (1)}$$

Note:
M = Percentage of male Betta (%)

Post-immersion and Post-rearing Survival Percentage

According to Rahmasari *et al*. (2021), post-immersion survival is calculated using the following formula:

$$SR = \frac{Nt}{N0}\ x\ 100\% \quad \text{..... (2)}$$

Note:
SR = Survival rate (%)
Nt = Number of viable fries or larvae at the end of immersion (individual)
N0 = Number of viable fries or larvae at the beginning of immersion (individual)

Water quality

The parameters measured in this research include the degree of acidity (pH), temperature, and dissolved oxygen (DO). These measurements were carried out descriptively and 4 times every 15 days during the research.

Data analysis

The data obtained in this study were analyzed using the ANOVA method. If the data showed a significant effect, further analysis was carried out using Duncan's advanced test at a significant level of 5% to determine the differences between treatments.

RESULTS

Percentage of Male Betta Sex

Based on the results of research that has been carried out, the highest percentage of betta was obtained in treatment P4, namely 77%; in treatment P3, 65%; in treatment P2, 58%; and the lowest percentage was obtained in treatment P1, namely 47%. Based on the results of the ANOVA test, it was found that immersion using bitter melon solution gave significant results (P>0.05) on the percentage of male betta. The percentage of male Betta can be seen in Figure 1.

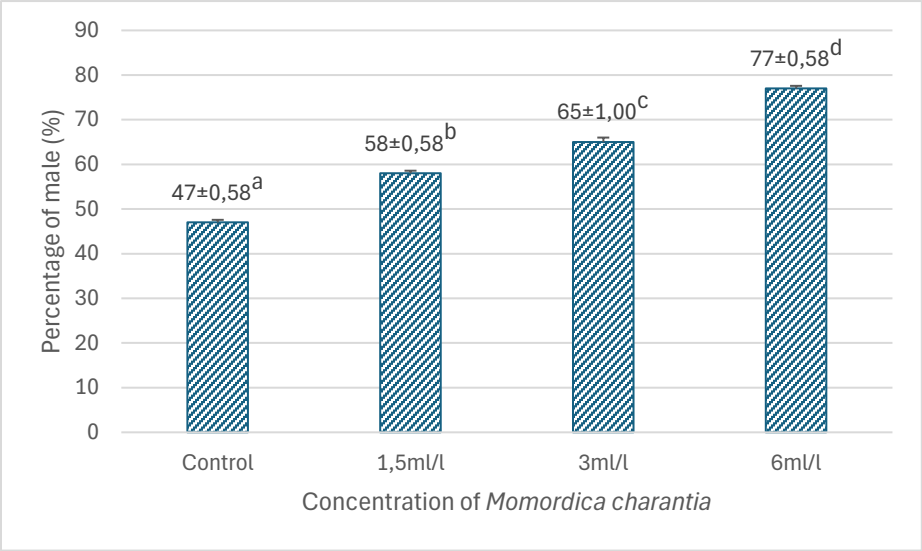

Figure 1. Percentage of Male Betta Sex. Note: different superscript letters indicate significant differences between treatments.

Post-Immersion Survival Rates

Based on the research results, it was found that the survival rate (SR) value of Betta larvae during immersion in treatments P1 (control), P2, P3, and P4 had a uniform value, namely 100%. The results of the ANOVA analysis showed that the immersion of bitter melon fruit solution in Betta larvae had no significant effect on the survival rate (SR) value of Betta during immersion. The results of measuring survival rates during immersion can be seen in Figure 2.

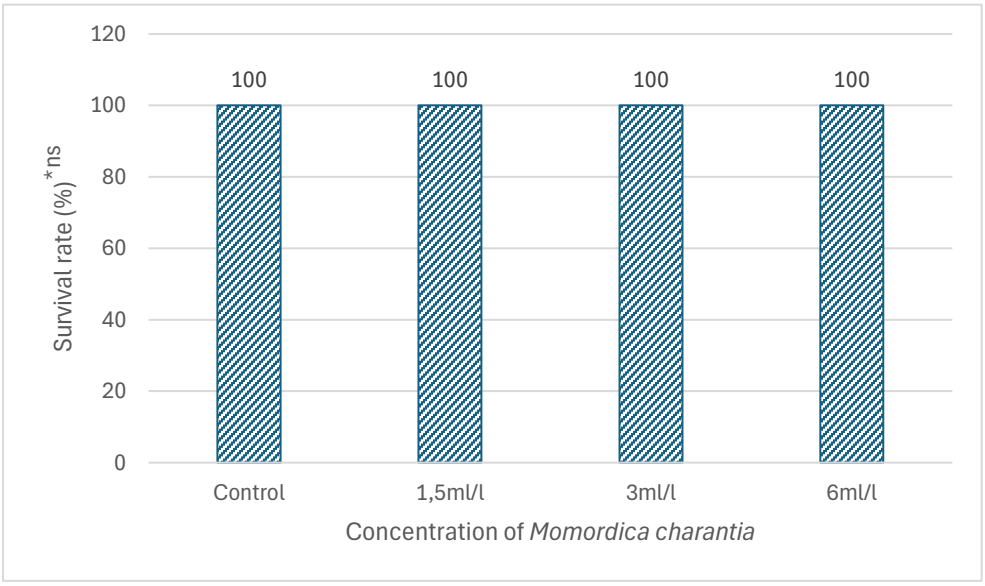

Figure 2. Post-immersion survival rate. Note: *ns indicate no differences between treatments

Post-Rearing Survival Rates

The survival rate (SR) value of Betta larvae during rearing in treatment P1 (control) and treatment P2 has the same value, namely 96.70%. The P3 treatment has an SR value of 95%, and the SR value of the P4 treatment is 93.33%. The results of the ANOVA analysis showed

that the immersion of bitter melon fruit solution in Betta larvae had no significant effect on the survival rate (SR) value of Betta during rearing. The results of survival measurements during maintenance can be seen in Figure 3.

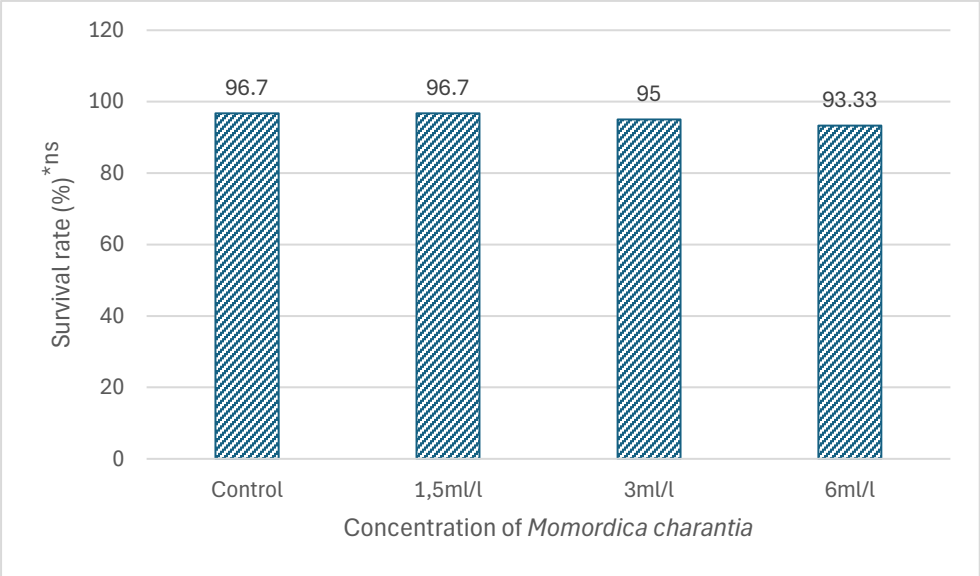

Figure 3. Post-rearing survival rate. Note: *ns indicate no differences between treatments

Water Quality

Water quality factors can influence the success of keeping Betta. The level of fish mortality is influenced by water quality. Bad water conditions can cause fish stress, resulting in the fish's appetite decreasing and causing death. So, water quality control must be maintained so fish can grow well.

Table 1. Water quality measurement results

Parameters	Unit	Value range	Optimal value range (Selfiaty et al., 2022)
Temperature	^{0}C	28.4-29	24-29
pH	-	7.5-7.9	6.5-7.2
Dissolved Oxygen	mg/l	5.2-6	>3

DISCUSSION

Percentage of Male Betta Sex

The percentage of male Betta in this study showed a significant increase in each treatment. The highest percentage of males was in treatment P4 with a concentration of 6 ml/l, namely 77%, and the lowest was in treatment P1 (control), namely 47%. The increase in the percentage of male Betta in the P4 treatment was influenced by the active ingredients in the bitter melon solution. According to Yuda *et al*. (2013), the results of phytochemical testing show that the active ingredients contained in bitter melon fruit extract include alkaloids, flavonoids, and saponins. The active compounds in bitter melon fruit that play an important role in directing the sex of Betta include flavonoids, saponins, and steroids.

The flavonoids in bitter melon are the main ingredients that play a role in directing the genitals of Betta. According to Mardiyanto *et al*. (2021), the secondary metabolite composition of bitter melon contains 27.34% flavonoids, 12.12% saponins, and 31% alkaloids. Sugiyanto & Anisyah (2022) stated that the highest total flavonoid content in bitter melon fruit extract was 17,702 mg/g, which was dried at a temperature of 100°C. This value is higher than the total flavonoids in celery and caramunting leaf extracts, which are used to determine the sex of Betta. According to Setyaningrum *et al*. (2021), the highest total flavonoids in celery extract was 1.466%, which was dried at a temperature of 40°C. Marwati *et al.* (2020) stated that the total flavonoids in caramunting leaf extract were 3.496%.

Flavonoid compounds have aromatase inhibitor properties, inhibiting the work of the aromatase enzyme; this enzyme catalyzes androgen (testosterone) into estrogen. Inhibition of the aromatase enzyme can cause androgens (testosterone) to increase so that the gonads form the male sex because the androgen hormone affects the gonads more. Increasing testosterone can reduce FSH and LH content, affecting spermatogenesis. Disruption of the spermatogenesis process also disrupts the resulting mortality. According to Winardi *et al*. (2021), inhibiting the aromatase enzyme causes a decrease in estrogen.

There was an increase in the percentage of male Betta as the concentration of the bitter melon solution increased to 6 ml/l. This increase is considered normal and has yet to reach the optimum limit for Betta. In line with Awaludin *et al*. (2019), adding celery extract up to 10 mg/L can increase the percentage of hickeys, while there is a decrease with an additional 40 mg/L dose. Using a dose that is too high can cause death, while a dose that is too low causes the fish to become sterile.

The increase in the percentage of male Bettas can be influenced by the dosage and immersion time used in the research. The dosage must be correct to produce optimum values in determining the sex of the Betta. P4 treatment by immersion of bitter melon fruit solution on Betta larvae with a concentration of 6 ml resulted in 77% male genitalia in Bettas. According to Awaludin *et al*. (2019), immersion Betta larvae with a concentration of 10 mg/L produced 73.33% male Betta. Bakar (2022) stated that immersion of Betta larvae at a concentration of 5 ml/l resulted in a male percentage of 68%. According to Winardi *et al*. (2021), immersion of pregnant mothers in guppy fish with a concentration of 2 ml/l of menstrual leaf extract resulted in a male sex percentage of 45.93%. Using a dose that is too low results in sex changes, resulting in less-than-perfect results. The difference in values can be caused by the total levels of the compounds contained therein. Another factor is the length of immersion; immersion applied using the bitter melon solution for 8 hours produces a male percentage of 77%. This value is higher than using celery extract and caramunting leaves. According to Awaludin *et al*. (2019), immersion of Betta larvae for 8 hours using celery extract resulted in a male percentage of 73.33%. Bakar (2022) explains that immersion caramunting leaf extract for 24 hours produces a percentage of % male Betta of 68%. According to Winardi *et al*. (2021), immersion of pregnant broodstock in guppy fish with an immersion period of 24 hours using menstrual leaf extract resulted in a male sex percentage of 45.93%. The difference in value can be influenced by the absorption capacity of the compound into Betta's body.

Other factors that can influence the masculinization of Betta include the administration time before going through the differentiation phase. This is in line with Selfiaty *et al.* (2022) that the success of masculinizing Betta can be influenced by the accuracy of sex determination or before passing the differentiation phase, immersion duration, immersion method, dosage, and materials used. Another factor that plays an important role in determining the success of fish masculinization is the environmental temperature during rearing. Temperature can

influence the number of males produced. The environmental temperature in the media tends to be low, so Betta offspring are directed to be female, while high temperatures can produce Betta offspring with male gender. According to Lubis *et al*. (2017), the formation of gonads in females is influenced by low temperatures, while high temperatures influence the development of gonads, leading to male genitalia.

Figure 4. Differences between male (a) and female Betta (b).

Sex differences in Betta can be observed directly through secondary sexual characteristics. Observing the sex of Betta can be done when the Betta is 60 days old. The physical characteristics of male Betta are more visually attractive than females. The color of the male Betta's body is more attractive, the fins are longer and wider, and the body shape is slimmer than the male Betta. According to Nugroho *et al*. (2020), the body color is relatively bright and slender compared to female Bettas; apart from that, the tail and anal fins are longer and wider in male Bettas, while females tend to be dimmer in color, slightly fatter and the fins are shorter.

Post-Immersion Survival Rates

Based on the research results, it was found that the survival value during immersion produced a percentage level of 100%. The high survival rate of Betta larvae during immersion shows that the immersion bitter melon fruit solution for Betta larvae is not dangerous or toxic for Betta larvae because the dose used during immersion is still relatively low so that the body of Betta larvae can tolerate it. Apart from that, the active ingredients in bitter melon act as antioxidants to ward off free radicals. According to Sudarsi & Rahmah (2018), the antioxidant test results of methanol extract from four types of bitter melon ranged from 79-88% at a concentration of 500 ppm per extract, while the bitter melon water was 82.05%. According to Bahagia *et al*. (2018), the flavonoid content in bitter melon acts as an antioxidant. Bermawie *et al*. (2020) stated that bitter melon contains 55% vitamin C for every 100 grams of bitter melon fruit; vitamin C is an antioxidant.

The survival rate during immersion is relatively high, influenced by various factors, including feeding according to the larva's mouth opening, food availability, and water cleanliness from leftover food or feces. Lubis *et al*. (2017) states that good maintenance techniques influence good survival. Another factor that influences the survival of Betta larvae is the length of immersion. The duration applied during immersion is still considered normal for Betta larvae, so it has no real effect on survival. According to Awaludin et al. (2019), immersion Betta larvae using celery extract for 8 hours resulted in a 63-100% survival rate. Ferdian *et al*. (2017) explained that immersion of Betta larvae using ginseng root extract for 8 hours resulted in a 79.17-100% survival rate. Bakar (2022) states that immersion of Betta larvae using cementing leaf extract for 24 hours results in a 95-100% survival rate. According

to Selfiaty *et al*. (2022), immersion Betta larvae produced 86% results using coconut water for 12 hours.

Post-Rearing Survival Rates

The survival obtained is still very good, ranging from 93.33-96.70%. Based on this value, it is influenced by good handling during maintenance, including proper feeding mechanisms and water quality management. Feeding Betta larvae in Artemia is sourced from natural food; this food plays an important role in increasing the growth of Betta larvae. According to Kaseger *et al*. (2019), providing natural food to larvae compared to artificial food can increase survival. Epram *et al*. (2021) stated that larvae-fed Artemia (100%) was 76.25% while Micro worm (100%) was 56.25%; this shows that feeding Artemia can improve fish growth better. Bettas that have reached fry size have different types of food adapted to their mouth openings. Betta fry is given natural food in the form of tubifex because this food can increase the survival of Betta. Furthermore, Epram *et al.* (2021) stated that tubifex is one of the best natural feeds for improving the survival of Betta. Agustini *et al*. (2022) noted that the increase in growth of Betta-given silkworms was higher than mosquito larvae because of the amount of food consumed; the body size factor of the food provided had to be smaller than the fish's mouth opening so that it was easier to prey on and digest the food.

Water quality is another factor that greatly influences the survival of Betta. Water quality includes temperature, pH, and DO. The water quality values obtained during maintenance are at the threshold for Betta tolerance, as shown in Table 3 above. According to Adriansyah (2020), fish survival can be influenced by physical and chemical factors, including competition, age, predators, parasites, density, and human handling. The survival of Betta during maintenance can be achieved by adding dry Ketapang leaves to maintain water quality. Samira (2021) states that ketapang leaves are one way to get a neutral pH, reducing the pH of the water by up to 16.5%. According to Waris *et al*. (2018), ketapang leaves can be used to maintain the health of Betta while maintaining broodstock, larvae, fry, and the hatching process. Apart from adding Ketapang leaves during maintenance, adding table salt plays a role in maintaining the health of Betta. Kaseger *et al*. (2019) stated that giving 5% table salt can maintain the health of larvae so that various diseases are environmentally friendly and not easily attack them.

The high survival rate during maintenance shows that giving bitter melon solution to Betta larvae is not dangerous because the dose used is relatively low, so the body of Betta larvae can tolerate it. Apart from that, the active ingredients in bitter melon fruit also act as antioxidants. The nutritional content in bitter melon fruit functions to increase the fish's immunity and ward off free radicals. According to Sudarsi & Rahmah (2018), the antioxidant test results of four types of bitter melon ranged from 79-88% at a concentration of 500 ppm for each extract, while the antioxidant test results for the water extract of bitter melon were 82.05%. According to Bahagia *et al*. (2018), the flavonoid content in bitter melon acts as an antioxidant. Bermawie *et al*. (2020) state that the vitamin C content in every 100 grams of bitter melon is 55%.

Water Quality

Based on the results of water quality research, it was found that the temperature ranged from 28.4-29°C. This water quality is good for the growth of Betta because it is still within the normal range. In line with Selfiaty *et al.* (2022), the optimal temperature for the survival of Betta ranges from 24-29°C. So, the temperature value during the research is the optimum range for Betta during maintenance. The degree of acidity (pH) is one factor that can influence the growth of Betta. The pH value during the study ranged from 7.5 to 7.9. The range

values obtained during the research were still tolerable for Betta. This pH range is neutral, so it is good for Betta. According to Selfiaty *et al*. (2022), the optimum pH range for the growth of Betta is between 6.5 and 7.2. The degree of acidity (pH) that is too high or too low can affect the growth of Betta; a pH that is too low can result in the death of the fish, which can be seen from irregular fish movements, swimming very fast on the surface of the water, the gill covers move actively, while pH conditions that are too high can causing stunted fish growth. Controlling and managing water quality is vital to stabilize it so it remains within the optimal range. Dissolved Oxygen (DO) plays an important role in supporting the growth of Betta. The dissolved oxygen values obtained during the research ranged from 5.4-6 mg/L. This value is still considered good for Betta. Selfiaty *et al*. (2022) stated that the good, dissolved oxygen value to support the growth of ornamental fish is >3 ppm. Factors that influence the high or low oxygen content in water are density, water temperature, and the level of oxygen consumption.

CONCLUSION

Based on the research that has been carried out, it can be concluded that the masculinization of Betta using the larval immersion method with different concentrations has a real influence on the percentage of male Bettas but has no real influence on the survival rate during immersion and rearing. In this study, immersion P4 treatment with a concentration of 6 ml/l increased the highest percentage of male Betta by 77%.

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