

Evaluation of Mangosteen Peel Extract Dosage on the Percentage of Male Guppy Fish (*Poecilia reticulata*)

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Abstract:

*This study aims to determine the best dose of mangosteen peel extract as a natural ingredient to increase the percentage of male sex in guppy larvae *Poecilia reticulata* aged less than 5 days. Mangosteen peel extract was prepared through maceration with 70% ethanol, then applied by immersion method for 2 hours at various doses of 1000; 1100; 1200; 1300; and 1400ppm along with one control without treatment, using a Completely Randomized Design with three replications. Parameters observed included the percentage of male-females, survival rate, and water quality (temperature and pH). The results showed that a dose of 1.4 ppm produced the highest male percentage of 57% and provided high survival, while a dose of 1 ppm produced the lowest male percentage. Xanthone compounds in mangosteen peel are thought to act as aromatase inhibitors that reduce estrogen formation thereby directing gonadal differentiation towards males. Thus, mangosteen peel extract at a dose of 1.4 ppm is effective as a natural masculinizing agent to increase the proportion of male guppies without reducing survival.*

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Introduction

Guppies (*Poecilia reticulata*) are a type of freshwater ornamental fish with high economic value in both domestic and international markets. Male guppies have a slender body, longer fins, and more vibrant colors than female guppies. (Matondang et al., 2018) Guppies are also easy to keep because they can thrive in a variety of water conditions and don't require

special care. The challenge in cultivating guppies is that the number of male fry produced is lower than the number of female fry.(Azrar et al., 2024)Therefore, efforts to increase the production of male guppy fry are crucial because male guppies are more attractive and have a higher market value.

Male guppies have more attractive morphological colors than female guppies, which makes them more expensive. Black Moscow guppies cost between Rp 20,000 and Rp 25,000 per fish, while female guppies cost between Rp 8,000 and Rp 12,000 per fish.(Azrar et al., 2024). The color of the male larvae has a wider tail fin with a variety of color patterns and has a very prominent red color compared to female guppy fish which only has one color (single color) and the physical size of the male larvae is smaller compared to the physical size of the female.(Finanta et al., 2020). The production of male fish needs to be increased in guppy cultivation by involving hormonal engineering.(Zidni et al., 2025)One method that utilizes sex reversal technology is masculinization.

Masculinization is a sex-reversal technology that can produce monosex fish by using hormonal engineering to change the sexual characteristics of females to males. Masculinization has been widely used in several ornamental fish species using various materials.(Aryoputro & Danakusumah, 2018)One ornamental fish frequently masculinized is the guppy (*Poecilia reticulata*) because it is a freshwater ornamental fish with high commercial value. Furthermore, based on their morphology, male guppies have slimmer bodies with brighter colors and fins than females. The more beautiful morphology of male guppies makes them a commodity with high appeal and sales potential, making breeding male guppies profitable.(Puspitha et al., 2023). Masculinization of guppy fish is carried out in the larval phase because at this stage, the male sexual organs are developing and can still be influenced by the hormone 17 α -methyltestosterone through the immersion method.(Aryoputro & Danakusumah, 2018).

The success of the masculinization method can be influenced by the type of hormone used, dosage, treatment time and application method. Generally, in masculinization of guppy fish, hormones are given, the hormone that is often used is the synthetic steroid hormone, namely 17 α -methyltestosterone.(Saputra et al., 2022). The use of the hormone 17 α -methyltestosterone has been banned because it is difficult to degrade and causes environmental pollution according to(Syarif & Winardi, 2021). Therefore, the use of alternative ingredients such as mangosteen peel extract can increase the percentage of males by 57% as was done in(Wibowo et al., 2024),using doses of 2.5 5.5 7.5 and 10 ppm, soaking was carried out for 5 hours.

Research has previously been conducted on administering mangosteen peel extract to produce more male fish than female fish, but this study used a different dosage. The goal of this study was to determine the optimal dosage for male-specific changes in fish larvae under five days old.

Research Methods

Research methods

This research began with the preparation of mangosteen peel extract using 400 grams of mangosteen peel. The mangosteen peel was peeled, dried in an oven at 60° Celsius for 24 hours, and ground using a blender to obtain the simplicia. 200 grams of simplicia was taken and homogenized with 1 liter of 70% alcohol and soaked for 72 hours. The maceration solution was

filtered using filter paper until smooth. The extraction filtration results used a rotary evaporator machine for 4 hours to separate the solution from the extract. Spawning was carried out by combining 1 male and 1 female broodstock. The test began with a lethal dose test with a dose variation from 1000 ppm to 2000 ppm. Death occurred at doses of 1500 ppm to 2000 ppm, so the research dose used was 1000 ppm to 1400 ppm.

Guppy larvae under 5 days old were soaked in a mangosteen peel extract solution with each dose. The larvae were soaked for 2 hours in a room away from direct sunlight. After soaking, the guppy larvae were transferred to a rearing tank. Water quality was checked three times during the rearing period.

This study used a completely randomized design (CRD) as an experimental quantitative method. The treatments used were mangosteen peel extract and an untreated control, with three repetitions. The treatment design was as follows:

1. Addition of 1000 ppm mangosteen peel extract
2. Addition of 1100 ppm of mangosteen peel extract
3. Addition of 1200 ppm of mangosteen peel extract
4. Addition of 1300 ppm of mangosteen peel extract
5. Addition of 1400 ppm of mangosteen peel extract
6. Without the addition of mangosteen peel extract (control)

Time and Place of Research

This research was conducted from August 15 to November 29, 2025, at the Fisheries Production and Entrepreneurship Development Laboratory, Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University.

Tools and materials

The tools and materials used in this study consist of tools and materials that support the research. The tools used include used gallons as maintenance containers, a sieve as a tool for taking fish, jars to hold extracts, filter paper as a separator for fine particles, a knife for cutting mangosteen peel, a sieve to separate liquids from solids, scales as a tool for measuring the weight of materials, an oven as a sample dryer, a rotary evaporator as a separator for extracts with solutions, sample bottles used as storage for extract results. In addition to these tools, other tools used are syringes to take liquids, measuring cups to measure volume, and labels to mark treatment containers.

The materials used in this study included guppy fish as the research subjects, small shrimp pellets as larval feed, mangosteen peel as the main extract, and 70% ethanol as the solvent. The tools and materials were selected for their high quality and used from the beginning to the end of the study.

Method of collecting data

The variables measured include the percentage of male fish and the percentage of female fish, and the survival rate. The water quality data measured includes temperature and pH.

1. Survival Rate(SR)

Survival is the percentage of fish that survive until the end of the study in a single container. According to Matondang et al., (2018), the formula for fish survival can be used:

$$SR = \frac{\text{Number of fish at the end of maintenance}}{\text{Number of fish at the start of maintenance}} \times 100\%$$

2. Percentage of Male and Female Fish

Calculating the percentage of male fish (j) and female fish (b) can be done using the following percentage formula:

$$J(\%) = \frac{\text{Number of male fish} \times 100\%}{\text{Number of samples}}$$

$$B(\%) = \frac{\text{Number of female fish} \times 100\%}{\text{Number of samples}}$$

3. Water Quality Parameters

The water quality parameters observed were temperature and pH. The instrument used for these measurements was a pH meter. Checks were conducted at the beginning, middle, and end of the study.

Data Analysis Methods

The obtained data were processed using Microsoft Excel 2019. The percentage ratio of male and female fish was analyzed using a normality test at a significance level of 0.05 and will be further tested using the Kruskal-Wallis test. All survival parameters were analyzed descriptively.

Results and Discussion

Based on Figure 1, the results of larval rearing carried out for 30 days showed the highest percentage of males at a dose of 1400 ppm. The lowest percentage of treatment results was at a dose of 1000 ppm. The significance results showed a significant difference in treatment 5 with a dose of 1400 ppm. The test was performed using the Kruskal-Wallis test, where the results for treatment 5 showed $P > 0.05$.



Picture 1. Male Percentage Graph

Mangosteen rind contains xanthenes, which act as natural aromatase inhibitors. Aromatase inhibitors work by inhibiting the production of estrogen, which is responsible for female sex development. The reduction in estrogen concentrations caused by aromatase inhibitors results in increased testosterone levels, which then lead to male sex development. (Berasa et al., 2023). This result is still low when compared with the research results Azrar et al., (2024) Masculinizing guppy fish using bitter melon solution with 5 ppm treatment resulted in a male ratio of 66%. The percentage increase reached 23% from without

giving bitter melon solution or control, which was 43% and increased in treatment 1 with the administration of 5 ml of bitter melon solution resulting in a percentage of 66% males in guppy fish because bitter melon contains active ingredients including alkaloids, flavonoids and saponins. These compounds play an important role in the direction of the sex of guppy fish. Flavonoids have the property of inhibiting the work of the aromatase enzyme, the enzyme functions to catalyze androgen (testosterone) into estrogen. Inhibition of the aromatase enzyme causes androgen (testosterone) to increase, so that the gonads form male sex because androgen hormones affect the gonads more. The effect of flavonoid content on the masculinization of guppy fish is also supported by research.(Safitri et al., 2023)which shows that the treatment of soaking guppy fish using mangrove honey is higher, namely 95% because mangrove honey has a higher flavonoid content compared to forest and livestock honey.

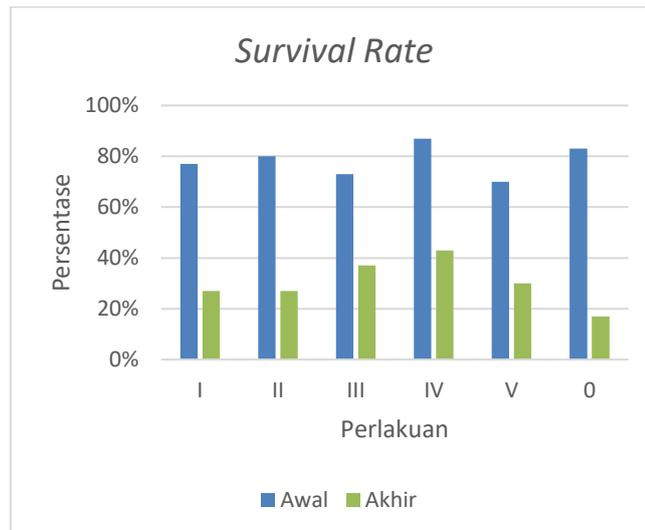
Based on Table 1, water quality observations conducted showed an average pH of 9.9 and an average temperature of 26.5°C. Water quality significantly influences the male percentage and the survival of fish larvae. Based on research by Awwalia et al (2025)Water quality has a significant impact on the metabolism and growth of fish larvae. Extreme increases or decreases in water quality can cause stress and affect the metabolism of fish larvae.

Table 1. Water Quality Data

No.	Water Quality	Measurement results
1	pH	9.9 ± 0.176
2	Temperature	26.5°C ± 0.396

pH, or the degree of acidity, is a description that indicates the acidity or alkalinity of the waters used in cultivation activities. In this study, pH measurements were carried out at the beginning, middle, and end for five repetitions, obtaining a value of 9.9 ± 0.176. The high pH value resulted from the accumulation of excess feed at the bottom of the water and increased the pH value in the water according to(Matondang et al., 2018)The influence of pH on the survival of guppies is crucial. The ideal water pH for guppies ranges from 6.6 ± 0.14, indicating optimal conditions for their survival. If the pH is too low or too high, it can cause stress to the fish and potentially lead to death. This unsuitable pH condition can disrupt the metabolic processes and overall health of the fish. Extreme or unstable pH conditions significantly affect the survival rate.(Sektiana et al., 2024).

The process of gender orientation can be achieved by manipulating environmental temperature. Temperature is a factor that can influence the speed of chemical reactions in the body, such as metabolic rate.(Arfah & Mariam, 2009)Environmental factors such as temperature influence the sex ratio in guppies (*Poecilia reticulata*). In this species, sex is determined by genetic factors, temperature levels, and genotype-temperature interactions. The sex ratio response to temperature is adjusted upward or downward, possibly by selection of sex-determining genes sensitive to higher or lower temperatures. Temperature influences primary sex determination during larval development. Most offspring produced at low temperatures early in the breeding season will be female, while most offspring produced at high temperatures occurring during the late breeding season will be male. However, in *Menidia Peninsula* fish, the sex ratio tends to be female at cool temperatures (11-19°C), while at fluctuating warm temperatures (17-25°C) the ratio is 1:1 or male-favored.(Shah et al., 2017).



Picture 2. Graphics Survival Rate

The survival rate of guppies among the five treatments was 87%, with treatment IV being the best, compared to the control treatment at 83%. The high survival rate of guppies during immersion in this study indicates that immersion using mangosteen peel extract is not toxic to guppies. Berasa et al., (2023) Treatment with mangosteen peel extract resulted in a survival rate of 82%, while black pepper extract resulted in a survival rate of 68%. This indicates that the use of mangosteen peel extract for guppy larvae is more optimal for fish survival because it is non-toxic to the larvae or the aquatic environment.

Conclusion and Recommendation

This study found that mangosteen peel extract at a dose of 1400 ppm produced the highest percentage of male guppies (*Poecilia reticulata*) at 57 percent in larvae less than 5 days old, with a survival rate of 87 percent, which was better than the control, making it the most effective natural masculinizing agent through 2-hour immersion. Xanthenes in the extract act as aromatase inhibitors that inhibit estrogen production, thereby directing gonadal differentiation towards males without significant toxicity, although this percentage is still lower than alternatives such as bitter melon or mangrove honey. However, the main limitation lies in the high water pH (9.9) due to feed accumulation, which deviates from the ideal range of 6.6-7.5 for guppies and may potentially affect larval metabolism, as well as the lack of complete statistical data such as initial sample size and replicates variation, which limits the generalization of the results.

As a practical implication, these findings support sustainable guppy farming with natural substitutes for the synthetic hormone 17 α -methyltestosterone, which is banned due to environmental pollution, thereby increasing the economic value of brightly colored male ornamental fish in the domestic and export markets. For further research, it is recommended to test dose combinations with optimal pH settings, compare longer immersion durations, and conduct molecular analysis of xanthone levels for a more precise mechanism, in order to

achieve a male ratio close to 80 percent and broader commercial application in fisheries laboratories.

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