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The Effectiveness of Betel (*Piper betle* Linn.) Leaf Extract in Controlling *Argulus* sp. in Koi Carp (*Cyprinus carpio*)

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ABSTRACT

Koi (*Cyprinus carpio*) are a type of ornamental fish with high economic value. The main problem faced by koi fish business operators and enthusiasts is infestation by the parasite *Argulus* sp. This study aims to determine the effectiveness of betel leaf extract in controlling *Argulus* sp. parasites in koi fish and to determine the most effective dose of betel leaf extract for controlling *Argulus* sp. parasites in koi fish. This study used a completely randomized design (CRD) with 4 treatments: Treatment K (control) with a betel leaf extract dose of 0 mL/L, Treatment 1 (P1) with a dose of 25 mL/L, Treatment 2 (P2) at a dose of 30 mL/L, Treatment 3 (P3) at a dose of 35 mL/L, and three replicates for each. The observed parameters included *Argulus* sp. mortality, fish survival rate (SR), behavioral response, and water quality. The results of the study showed that betel leaf extract had a significant effect ($p<0.05$) on the mortality of *Argulus* sp., with the highest value observed in P3 at a dose of 35 mL/L (98%), while the highest survival rate was obtained in P2 at a dose of 30 mL/L (93.33%).

INTRODUCTION

Koi (*Cyprinus carpio*) are a type of ornamental fish with high economic value. National koi production in the first quarter of 2023 totaled 102,261.75 fish, exceeding the production target of 96,948.91 fish (DJPB, 2023). Koi, also known as nishikigoi, are highly sought-after ornamental fish. This is because they possess striking physical beauty, characterized by their varied body colors, and are also believed to bring good luck to anyone who keeps them.

One of the main problems faced by koi fish business owners and enthusiasts is when their fish are attacked by parasites (Susanti *et al*., 2022). Fish affected by parasites experience stress. This weakens the fish's immune system, making them more susceptible to disease. Stress in fish can also lead to increased parasite reproduction, making the fish even more vulnerable due to the large number of parasites growing on them (Ramadhan & Sari, 2019).

Field observations indicate that farmed koi are susceptible to parasites, including *Argulus* sp., commonly known as fish lice. A study on parasite identification in farmed koi fish found the presence of *Argulus* sp. as one of the parasite species infesting the fish's body, although its prevalence is relatively lower compared to other parasites, such as *Dactylogyrus*

sp. (Hidayanti *et al*., 2025). Infection by *Argulus* sp. can cause lesions on the host's skin, which in turn opens the door to secondary infections by other pathogens and induces physiological stress that negatively impacts the growth and survival of farmed fish.

Parasite infestations can be managed through both preventive measures and treatment. Preventive measures include controlling water quality to match the fish's natural habitat and providing feed that is appropriate in both quality and quantity. Meanwhile, disease management through treatment can be carried out using chemicals or antibiotics. Some chemicals commonly used by aquaculturists do not break down easily in nature, so they are classified as environmentally unfriendly. One alternative is the use of traditional plants that inhibit the growth of microbes and fungi, such as betel leaves (Ghofur *et al*., 2017).

Betel leaf (*Piper betle* Linn.) is a natural ingredient with potential antifungal and antibacterial properties, as it contains essential oils rich in cavicol, an antiseptic believed to inhibit the growth of parasites (Pradhan *et al*., 2013). Furthermore, (Syahida *et al*., 2013) reported that 82.8% of the components of betel leaf essential oil consist of phenolic compounds, while only 18.2% are non-phenolic compounds.

Several studies on the use of betel leaf in fish disease control have been conducted. Betel leaf extract has been reported to be effective against pathogenic bacteria in fish (Bond *et al*., 2019; Mulia & Husin, 2012).

Research findings (Laoli *et al*., 2024) indicate that natural substances, such as plant extracts, essential oils, and other bioactive compounds, have significant potential as antimicrobial agents for treating freshwater fish diseases. These substances are not only effective at inhibiting the growth of fish pathogens but also safer for the environment and human health than synthetic chemicals and antibiotics. However, this study has not specifically tested the effects of betel leaf extract on koi fish; therefore, further research is needed regarding its effectiveness and optimal dosage against ectoparasites in koi fish.

METHODS

Time and Location

This study was conducted from April 29 to May 14, 2025, at UD. Andar Ikan Hias, Peteluan Indah Village, Lingsar Subdistrict, West Lombok Regency.

Equipment and Materials

The equipment used in this study included 10-liter jars, an aerator, water quality measuring instruments (pH meter, thermometer, DO meter), a microscope, a loop, a hemocytometer, an analytical balance, a blender, beakers, a hot plate, a sieve, measuring cups, a ruler, and a millimeter block. The materials used included koi fish measuring 9–10 cm, *Argulus* sp., 500 grams of green betel leaves, 2 L of 96% ethanol, 1 L of distilled water, NaCl solution, and filter paper.

Research Design

This study employed an experimental method using a Completely Randomized Design (CRD). The experiment consisted of four treatments with three replicates per treatment. The treatments applied in this study were modified from the research by (Abiyoga *et al*., 2021). The treatments in this study are as follows:

Treatment K (control) with betel leaf extract dosage
Treatment 1 (P1) with a dosage of 25 mL/10 L
Treatment 2 (P2) with a dosage of 30 mL/10 L
Treatment 3 (P3) with a dosage of 35 mL/10 L

This study began with the preparation of 10-liter containers, their maintenance, and the installation of an aeration system. The koi fish fry used were obtained from UD. Andar Ikan Hias were confirmed to be healthy and free of *Argulus* sp.

The preparation of betel leaf extract was carried out based on a modified method (Hasan *et al*., 2016). The process began with the selection of green leaves, followed by washing, cutting, sun-drying, and grinding into a fine powder. Five hundred grams of the powder were dissolved in 5 L of distilled water, heated to 50°C for 15 minutes, then filtered and stored. The extract's efficacy was tested by placing 20 *Argulus* sp. into each jar containing koi fish and leaving them for 24 hours. Afterward, the fish were immersed in the betel leaf extract at the appropriate dosage for 1 hour while their behavior was observed. The water was then filtered to count the number of *Argulus* sp. that died or fell off. Following the treatment, the water was replaced, and the fish were observed again for 24 hours. Fish health parameters, *Argulus* sp. mortality, as well as temperature, pH, and dissolved oxygen, were measured daily as indicators of treatment success and safety.

Data Analysis

Fish Health/Behavior

Fish health data were analyzed descriptively based on observations of the fish's behavior and physical condition during the study.

Mortality Rate of *Argulus* sp.

The mortality rate of *Argulus* sp. can be calculated using the formula (Putriningtias *et al*., 2022).

$$\text{MR}\ (\%) = \frac{\text{Nt}}{\text{N0}} \text{x}\ 100$$

Where:

MR : Mortality Rate (%)
N0 : Number of *Argulus* sp. at the start of the observation
Nt : Number of *Argulus* sp. at the end of the observation

Fish Survival Rate

The survival rate was calculated using the formula (Effendi, 1979) cited in (Putriningtias *et al*., 2022).

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

Where:

SR : Survival Rate (%)
Nt : Final number of fish (individuals)
N0 : Initial number of fish (individuals)

Water Quality

Water quality is a crucial factor supporting the growth and development of aquatic organisms. Therefore, the provision of an optimal environment significantly affects the success of the cultivation of these organisms. During the research, water quality monitoring was carried out periodically every 10 days. The observed parameters included temperature, pH, and dissolved oxygen (DO), which were measured using a thermometer, pH meter, and DO meter, respectively.

Analysis of Variance

This study employed a completely randomized design (CRD), and the significance of the results was assessed using ANOVA (Analysis of Variance). The purpose was to determine whether there were significant differences between the means of two or more groups. If the ANOVA results show that the calculated F value is greater than the critical F value ($p < 0.05$),

it can be concluded that there is a significant difference between the means of those groups, and Duncan's post-hoc test is conducted to examine differences between treatments.

RESULTS

Fish Health Status

Fish health status describes the fish's external condition, as evidenced by their physical characteristics and behavior during the study. The condition of fish infested with *Argulus* sp. is shown in Figure 1. The results of observations on the health status of the test fish after treatment with betel leaf extract are presented in Table 1.

Figure 1. Condition of Fish Infected by *Argulus* sp.

Table 1. Health and Behavioral Conditions of Fish

Treatment	Before Treatment	1 Hour During Treatment	24 Hours After Treatment
K	- Active fish movement, - Good feeding response, - Normal gill cover	- Normal fish movement, - Normal gill cover movement	- Fish jumping, - Rubbing their bodies against the tank, - Some fish appear to have sores
P1	- Active fish movement, - Good feeding response, normal gill cover	- Rapid fish movement - Rapid gill cover movement	- Normal fish movement, - Normal gill covers, - Good feeding response
P2	- Active fish movement, - Good feeding response, - Normal gill cover	- Rapid fish movement, - Rapid gill cover movement	- Normal fish movement, - Normal gill covers, - Good feeding response
P3	- Active fish movement, - Good feeding response, - Normal operculum	- Rapid fish movement - Rapid gill cover movement, - Some fish move at an angle	- Fish move quickly, - Gill covers moves rapidly, - Poor response to feed

Mortality of *Argulus* sp.

Mortality is a measure of the death rate among individuals in a population. Mortality of *Argulus* sp. refers to the number of *Argulus* sp. that died after being administered betel leaf extract. The results of the mortality observations for *Argulus* sp. are shown in Figure 2.

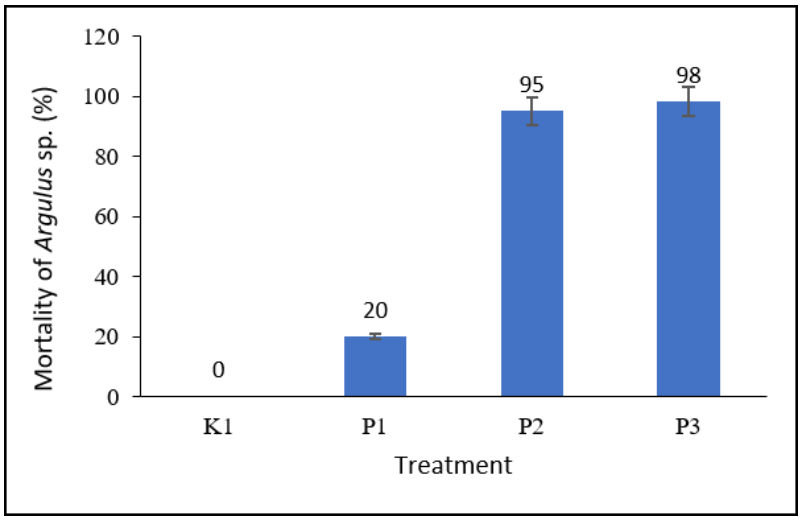

Figure 2. Mortality of *Argulus* sp. After 1 Hour of Soaking in Betel Leaf Extract

Survival Rate (SR)

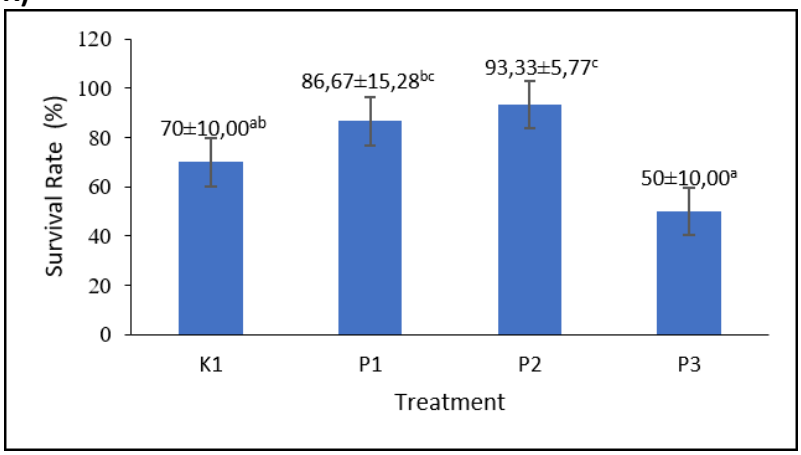

Figure 3. Survival Rate After 24 Hours of Soaking

Water Quality

Water serves as the medium in which fish are raised and as their habitat.

Table 2. Water Quality Measurement Results

Parameter	Measurement Results	Optimal Range	Reference
DO (mg/L)	6.4–7.0	>5	(Badan Standardisasi Nasional, 2019)
pH	7.4–7.7	6.5–8.5	(Badan Standardisasi Nasional, 2019)
Temperature (°C)	27–27.6	24–29	(Badan Standardisasi Nasional, 2019)

DISCUSSION

Fish Health Status

Figure 1 shows fish infected with *Argulus* sp., typically exhibiting lesions on their bodies. These wounds are indicative of infection by the pathogen (*Argulus* sp.) attacking the test fish. This aligns with research Steckler & Yanong (2013), which found that bleeding in fish due to *Argulus* sp. infestation is caused by irritation from the stylet (sucker). Based on the observations conducted, there are specific characteristics and behaviors exhibited by koi fish

when infected with the pathogen. Visible external signs on the body include ulcerative lesions that cause the scales, skin, and flesh of the koi to appear open, raw, and as if bleeding. A lack of aggressive and active movement, unlike that of healthy fish, indicates the fish is in a sick state. They frequently bump themselves against the sides of the tank due to feeling discomfort. This aligns with research Azmi *et al*. (2013); Kumari & Nomani (2021), where fish infected with *Argulus* sp. exhibit behavioral abnormalities, such as erratic swimming, rubbing their bodies against the tank walls, and swimming toward the aeration system.

Table 1 shows that the behavior of fish after being immersed in betel leaf extract is nearly identical to that of healthy fish. However, when immersed in betel leaf extract, the fish exhibited faster gill cover movements compared to healthy fish. The decrease in dissolved oxygen levels caused the fish to accelerate their gill cover movements in order to rapidly obtain oxygen to meet their respiratory needs.

After 24 hours of observation, many fish were still alive, and some had died, and this was true for nearly all treatment groups. However, the group treated with betel leaf extract appeared more active.

Mortality of *Argulus* sp.

As shown in Figure 2, the highest mortality rate was observed in treatment P3, with a mortality rate of 98% for *Argulus* sp., followed by P2 at 95%, and P1 at 20%, while the lowest mortality rate was found in the control (K) treatment at 0%. These results indicate that the population of the ectoparasite *Argulus* sp. decreases as the dose of betel leaf extract administered increases. This suggests that the addition of betel leaf extract is effective in controlling *Argulus* sp., with higher doses of the extract resulting in a greater number of *Argulus* sp. deaths.

Betel leaf essential oil contains phenolic compounds that play a role in damaging the cell structure of the ectoparasite *Argulus sp.* through mechanisms of protein denaturation and coagulation, as well as the dissolution of lipid components in the cell membrane. This damage can lead to impaired membrane permeability, leakage of cellular contents, growth inhibition, and ultimately the death of the parasite. The higher the concentration of phenols in the extract, the greater its effectiveness in controlling parasites, including *Argulus* sp.

This is consistent with the findings of Fachriyah *et al*. (2023); Ghofur *et al*. (2017), who reported that phenol, the dominant component in the essential oil of *Piper betle* L. exhibits antimicrobial activity, with higher phenol levels corresponding to increased antioxidant activity. Furthermore, phenols and their derivatives can interact with the cell walls of microorganisms, thereby triggering protein denaturation and coagulation. This process causes proteins to lose their physiological functions and prevents them from performing their roles optimally (Lobiuc *et al*., 2023). Additionally, phenolic compounds in betel leaves can inhibit and kill ectoparasites in fish, such as *Dactylogyrus, Gyrodactylus*, and *Argulus* sp. (Susanti *et al*., 2022).

The results of the Analysis of Variance (ANOVA) test indicate that soaking koi carp (*Cyprinus carpio*) in betel leaf extract had a significant effect on the number of dead *Argulus* sp. This is indicated by a calculated F-value greater than the critical F-value ($p < 0.05$). Thus, it can be concluded that soaking with betel leaf extract has a significant effect on the mortality rate of *Argulus* sp. To determine the significance between treatments, a post-hoc Duncan's test was conducted. The results showed that treatment P3 was not significantly different from treatment P2, but was significantly different from P1 and K (Control). These results indicate that treatment P3, with a betel leaf extract dose of 35 ml/L, is the most effective treatment for controlling *Argulus* sp.

Generally, one of the measures that can be taken to prevent pathogens from entering and disrupting the lives of farmed fish is to manage the environment and the activities carried out, such as providing feed according to their needs, so that it does not affect the water quality, which serves as the fish's living environment. However, these activities must be accompanied by the addition and administration of substances that can improve fish health, specifically those containing immunostimulants that can stimulate the fish's immune system, thereby making them resistant to pathogens.

Survival Rate (SR)

A high survival rate indicates the success of a given aquaculture operation. As shown in Figure 3, the highest survival rate was observed in treatment P2, with a survival rate of 93.33%. The high survival rate is attributed to the active compounds acting as immunostimulants, enabling the fish to survive when exposed to external disturbances. Meanwhile, the low survival rate in treatment P3 was due to an excessively high dose of betel leaf extract, causing the test fish to experience respiratory distress as oxygen levels in the test tank decreased during the treatment. This aligns with Mustapha *et al*. (2020), who stated that high treatment doses carry the risk of causing fish toxicity, such as behavioral changes, gill tissue damage, and death. The active compounds contained in betel leaves also have specific characteristics. When present in excessively high concentrations within a substance, these compounds can be toxic to the fish themselves but possess an excellent ability to reduce pathogens. According to Muahiddah & Alim (2023); Susanti *et al*. (2022), larval survival rates are influenced by the dosage and duration of larval immersion. An appropriate dose and the correct immersion duration will result in a high percentage of larval survival. Conversely, if the dose and immersion duration are not appropriate, the larval survival rate will decrease. Prolonged immersion of the larvae will cause them to become stressed and render the fish sterile, leading to larval mortality.

Water Quality

DO (Dissolved Oxygen) refers to the concentration of dissolved oxygen in the water where fish live. The water must supply dissolved oxygen in accordance with the needs of the farmed fish. The dissolved oxygen levels required vary by fish species and by fish size. In this study, dissolved oxygen levels ranged from 6.5 mg/L to 7.0 mg/L. The dissolved oxygen levels obtained fall within the normal range. Water bodies suitable for aquaculture should have a dissolved oxygen concentration of at least 5 mg/L. According to the Badan Standardisasi Nasional (2019), The minimum recommended dissolved oxygen concentration for aquaculture is 5 mg/L. A dissolved oxygen concentration below 5 mg/L can have adverse effects on all aquatic organisms (Kamil *et al*., 2024).

pH (acidity level) refers to the state and condition in which water can be acidic (<7), alkaline (>7), or neutral (=7). The pH level is influenced by temperature, uneaten feed, and the metabolism of the cultured organisms. If aquaculture water is not treated frequently, the pH value will approach acidic (pH < 7) and/or alkaline (pH > 7). Such values can cause stress in fish and, over time, lead to their death due to toxicity. In this study, the pH values obtained ranged from 7.4 to 7.7. The pH values obtained fall within the normal pH range for koi fish. This aligns with the Badan Standardisasi Nasional (2019), which states that the ideal pH range for koi fish farming is between 6.5 and 8.

Temperature is an indicator of water conditions, meaning whether the water is warm or cold. In other words, it describes the environmental conditions of the water in terms of warmth and coldness. Water temperature is greatly influenced by light (sunlight). In aquaculture, water temperature is closely related to the rate of chemical processes within the

fish's body. High temperatures can accelerate chemical processes within the body, such as osmosis (osmoregulation), food absorption, and other chemical activities in the fish's body; thus, if the temperature is too high, it can cause damage to the fish's internal organs due to rapidly triggered chemical processes occurring within the body. If water temperature is low (cold), chemical processes within the fish's body may slow down, resulting in reduced metabolic activity that leads to stunted growth (small or dwarf fish). In this study, the measured water temperature ranged from 27–27.6 °C. The recorded temperatures remain within the normal range for koi fish maintenance and cultivation activities. This aligns with Kamil et al. (2024), which states that koi can survive even in poor-quality water. The optimal or ideal temperature for koi, according to the Badan Standardisasi Nasional (2019) is 24–29°C. Although koi can survive at certain temperatures, drastic temperature changes can adversely affect them, leading to stress and mortality.

CONCLUSION

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

1. Betel leaf extract is effective for controlling *Argulus* sp. populations in koi fish.
2. The most effective dose of betel leaf extract for controlling *Argulus* sp. and ensuring optimal survival rates in koi fish is 30 mL/L.

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