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Research Article

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Effect of Papaya (*Carica papaya*) Leaf Extract on the Persistence and Burden of Argulus Infestation in Comet Fish (*Carassius auratus*)

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ABSTRACT

Papaya (*Carica papaya*) leaf extract was evaluated as an environmentally friendly and cost-effective phytotherapeutic agent for controlling *Argulus* infestation in comet fish (*Carassius auratus*). The extract (100 ppt) was prepared by boiling papaya leaves at 90 °C for 15 min. A 30-day experiment was conducted using a completely randomized design with four treatments and three replicates, corresponding to extract concentrations of 0 ml L^{-1} (A), 7 ml L^{-1} (B), 14 ml L^{-1} (C), and 21 ml L^{-1} (D). To induce infection, each aquarium was inoculated with 10 *Argulus* individuals for four days prior to treatment. Fish were then immersed in the respective treatments for 60 min and monitored for seven days. Immersion in papaya leaf extract significantly reduced *Argulus* infestation, with the highest parasite detachment observed at 21 ml L^{-1} (70%), which was significantly greater than lower-dose treatments ($P < 0.05$). Survival rates remained high in all treated groups (100%), whereas the control exhibited lower survival (86.67%). Treated fish showed marked clinical improvement, including reduced mucus secretion and progressive lesion healing. Parasite detachment increased in a clear dose-dependent manner, from 0% in the control to 16.67% (B), 33.33% (C), and 70% (D) in treatments, respectively. Papaya leaf extract, particularly at 21 ml L^{-1}, is a promising phytotherapeutic option for managing *Argulus* infestation in ornamental fish culture. Further studies should explore long-term efficacy and application at production scale.

Keywords: *Argulus,* carpain, comet fish, papaya leaf, detachment

ABSTRAK

Ekstrak daun pepaya (*Carica papaya*) dievaluasi sebagai agen fitoterapi yang ramah lingkungan dan hemat biaya untuk mengendalikan infestasi *Argulus* pada ikan komet (*Carassius auratus*). Ekstrak (100 ppt) disiapkan dengan cara merebus daun pepaya pada suhu 90 °C selama 15 menit. Penelitian ini dilaksanakan selama 30 hari menggunakan rancangan acak lengkap dengan empat perlakuan dan tiga ulangan, yaitu konsentrasi ekstrak 0 ml L^{-1} (A), 7 ml L^{-1} (B), 14 ml L^{-1} (C), dan 21 ml L^{-1} (D). Untuk menginduksi infeksi, setiap akuarium diinokulasi dengan 10 individu *Argulus* selama empat hari sebelum perlakuan. Selanjutnya, ikan direndam dalam perlakuan masing-masing selama 60 menit dan diamati selama tujuh hari. Perendaman menggunakan ekstrak daun pepaya secara signifikan menurunkan tingkat infestasi *Argulus*, dengan pelepasan parasit tertinggi terjadi pada konsentrasi 21 ml L^{-1} (70%), yang secara signifikan lebih tinggi dibandingkan perlakuan dosis lebih rendah ($P < 0,05$). Tingkat kelangsungan hidup ikan tetap tinggi pada seluruh kelompok perlakuan (100%), sedangkan kontrol menunjukkan nilai yang lebih rendah (86,67%). Ikan perlakuan memperlihatkan perbaikan kondisi klinis yang nyata, ditandai dengan penurunan produksi lendir berlebih dan penyembuhan lesi secara bertahap. Tingkat pelepasan *Argulus* meningkat secara jelas seiring peningkatan dosis, dari 0% pada kontrol menjadi 16,67% (B), 33,33% (C), dan 70% (D). Ekstrak daun pepaya, khususnya pada konsentrasi 21 ml L^{-1}, berpotensi sebagai alternatif fitoterapi untuk pengendalian infestasi *Argulus* pada budidaya ikan hias. Penelitian lanjutan perlu dilakukan untuk mengevaluasi efektivitas jangka panjang dan penerapannya pada skala produksi.

Kata kunci: Argulus, carpain, daun pepaya, ikan komet, pelepasan.

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1. Introduction

The comet fish (*Carassius auratus*) is an ornamental fish that originates from China (Salim et al., 2023). According to data from the Indonesian Ministry of Marine Affairs and Fisheries, KKP (2022), the total production volume of ornamental fish in Indonesia is substantial, reaching 1.47 million in 2022. The price of comet fish ranges from IDR 5,000-IDR 10,000 depending on the size, type, and color (Umalekhay et al., 2020). Comet fish are freshwater ornamental fish that are widely favored because of their appealing color patterns, attractive body shapes, and relatively simple maintenance requirements (Rosid et al., 2019; Udjan et al., 2023). This ornamental fish contributes to the Indonesian economy, as it has great sell value and high demand in both domestic and global markets, attracting many businessmen to cultivate comet fish (Putri et al., 2024).

Fish as part of living organisms, is inevitable with disease during their lifetime, which will attack them and present negative effects. The effects of fish diseases not only occur only at a household level, but also the whole process of fish farming activity (Peeler et al., 2023). Relating to the One Health concept, the disease could also impact to the surrounding environment due to the transfer of pathogens to aquatic ecosystems, so the management of fish farming must be put intention (Johansen, et al., 2011). Moreover, the aquatic diseases also could affect people health as humans use the aquatic environment for a wide-range of purposes (Taylor et al., 2011). Thus, the study of aquatic animals' pathogens is crucial to prevent the outbreak of aquaculture diseases.

While varying pathogens are possible to invade fish, parasitic infections are commonly found in ornamental fish cultivation and represent a major obstacle to successful fish cultivation. Fish infected by parasites experience downgrading of quality and the final effect of death, which affects the overall sale in the market (Ma'arif et al., 2024). Notably, comet fish are susceptible to infestation by the ectoparasite *Argulus*, a genus of fish lice known to infect freshwater species (Alom et al., 2019). The presence of these parasites in host fish can result in a range of adverse symptoms including injuries, bleeding, abnormal swimming patterns, poor feeding, increased rubbing behavior, and excessive mucus secretion (Juwahir et al., 2016). The disease caused by this parasite is called argulosis, which leads to secondary infections in its hosts, lower reproduction rates, and death in fish (Parida et al., 2018).

Current treatment approaches often involve chemical agents, such as organophosphates, potassium permanganate, and dimilin (Das et al., 2018; Gultom et al., 2018; Thakur et al., 2023). However, the expenses and potential environmental harm associated with these chemicals (Alom et al., 2019) highlight the need for more sustainable solutions. Utilizing alternative, natural medicinal resources offer a promising avenue due to their low cost, environmental friendliness, and ease of acquisition (Azizah et al., 2019; Thakur et al., 2023).

Given the drawbacks of chemical treatments, exploring alternative solutions, such as papaya leaf extract, shows promise for controlling Argulus parasites. Previous research has indicated the efficacy of papaya leaf extract (Kismiyati et al., 2012), resulting 88% *Argulus* infestations removal from comet fish after immersion in 30% papaya leaf juice for 20 min. Other studies by Harahap et al. (2021) achieved 80% *Argulus* mortality and 100% fish survival in koi, and Azizah et al. (2019) reporting 75.56±7.70% parasite mortality and 86.67±11.5% fish survival in goldfish (*Cyprinus carpio*) using extracts prepared via infundation support papaya leaf extract has great potential as an innovative treatment in *Argulus*-infested fish. The infundation method is particularly appealing to farmers because of its simplicity, speed, ease of use, and low cost (Yohanes et al., 2018). Acknowledging these limitations, this study proposes an alternative solution that utilizes papaya leaf extracts.

Epidemiological strategies that focus on prevention, sustainable management, and lowering pathogen prevalence, the use of herbal-based interventions is gaining wider attention. Extracts from papaya (*C. papaya*) leaves contain bioactive constituents that interfere with parasite metabolic processes and ultimately induce mortality. However, the optimal and most effective dosage levels remain to be fully investigated and established. To further investigate this potential, this study aimed to specifically evaluate the effectiveness of soaking in papaya leaf extract via the infundation method, and to explore new dosages to identify the most effective method for controlling *Argulus* infestation in comet fish.

2. Materials and methods

2.1. Preliminary Test – Argulus Behavior

Preliminary tests were carried out to observe the behavior of *Argulus* when exposed to papaya leaf extract in the absence of fish, ensuring that the direct effects of the extract on the parasite could be assessed. Three *Argulus* individuals were used to determine an appropriate test concentration, with parasites exposed to papaya leaf extract at 7, 14, and 21 ml L^{-1} for 60 minutes. During exposure, the parasites were placed in petri dishes and examined under a microscope using the Motic Images Plus application to observe changes in morphology and anatomical integrity. Behavioral observations focused on swimming patterns, including the parasite's approach behavior toward a host, the time required to reach the host, and the occurrence of flushing movements (rapid, uncontrolled vertical motion). In addition, preferred attachment sites on the body surface of comet fish were documented to provide a clearer understanding of host–parasite interaction dynamics.

2.2. Phytochemical Test

Phytochemical profiling of the various *C. papaya* extracts was performed to verify the occurrence of key bioactive compound groups. The assessment employed differential solubility assays, characteristic colorimetric reactions, and reagent-induced precipitation to confirm the presence of targeted chemical families. The phytochemical tests conducted included tests for tannins, alkaloids, saponins, and flavonoids. Phytochemical analysis was performed on the papaya leaf extract produced using the infundation method. The tests included saponins were detected based on their inherent foam-forming properties Majinda 2021; Harahap et al., 2021), alkaloids were identified using classical precipitation assays involving Mayer's and Dragendorff's reagents (Adejoke et al., 2019; Yohanes et al., 2018), the presence of flavonoids was established through the cyanidin reaction, which produces a diagnostic color change (Biesaga, 2011), and tannins were confirmed through the characteristic color complex formed with ferric chloride (de Hoyos-Martínez et al., 2019; Alifah et al., 2023). These tests were performed at the Aquaculture Laboratory of the Faculty of Fisheries and Marine Science, Universitas Diponegoro.

2.3. Container Preparation

The experimental system consisted of a thoroughly sanitized glass aquarium (80 × 40 × 40 cm). After cleaning, the unit was air-dried under direct sunlight to minimize potential microbial contamination, then filled with 5 L of dechlorinated freshwater to establish a controlled environment for subsequent procedures.

2.4. Fish Preparation

Sixty comet fish (mean length 4.83 ± 0.20 cm; mean weight 1.30 ± 0.12 g) were used in this study. Before the experiment, fish were fasted for 24 h for acclimation and subsequently maintained for seven days under controlled conditions to ensure uniform health status and suitability for experimentation (Rejeki et al., 2019; Asni et al., 2020). During acclimation, fish were fed to apparent satiation twice daily, once in the morning and once in the evening.

2.5. Parasite Preparation

One hundred and twenty *Argulus* parasites were prepared for the experiment, with a target density of 10 parasites per aquarium (Gultom et al., 2018). These parasites were collected over seven days from naturally infected fish obtained from Pasar Raya Salatiga. *Argulus* was carefully removed from the fish using tweezers and maintained in a separate aquarium with several fish as hosts. The detachment of *Argulus* parasites from comet fish after soaking in papaya leaf extract was quantified by tracking the number of parasites that detached from an initial attachment of 10 per fish.

2.6. Papaya Leaf Extraction

Papaya leaf extract was prepared using 100 g of fresh papaya leaves per liter of water via the infundation method (Oktavia et al., 2020). One liter of water was heated to 90°C, and the washed papaya leaves were added and stirred. The extract was filtered after 15 min of infusion.

2.7. Main Research

Following the preparation of the papaya leaf extract, a Completely Randomized Design (CRD) was implemented with four treatments and three replications to assess its effectiveness across varying doses. The treatments were A (0 ml L^{-1} papaya leaf extract), B (7 ml L^{-1}), C (14 ml L^{-1}), and D (21 ml L^{-1}). On the 8th day of the experiment, the fish were infected with *Argulus*. Clinical symptoms were observed for four days following infection (Azizah et al., 2019). Subsequently, on the 13th day, the fish in each treatment group were soaked in the respective papaya leaf extract doses for 60 min, with each treatment replicated three times in 3 L jars. A 7-day recovery period followed the soaking treatment, during which fish were observed.

2.8. Clinical symptoms

Clinical manifestations were assessed through direct visual monitoring conducted prior to *Argulus* exposure, throughout the course of infection, and following immersion in papaya leaf extract. The evaluation encompassed changes alterations in swimming performance and visible morphological abnormalities exhibited by the experimental fish.

2.9. Survival Rate (SR) of Comet Fish

The survival rate was calculated as the percentage of fish surviving at the end of the experiment relative to the initial number of fish stocked in each aquarium. The formula was applied as follows:

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

Where:

SR = Survival rate

Nt = number of individuals alive at the end of a time period

N0 = number of individuals at the start of the period

2.10. Detachment of *Argulus*

The detachment rate was assessed by counting the number of parasites that were no longer attached to the host. The calculation followed the method described by Haditomo et al. (2025).

$$P = \frac{a}{b} x\ 100\% \quad \text{(ii)}$$

Where:

P = Detachment parasite percentage

a = Detachment parasites

b = total parasites

2.11. Water Maintenance and Quality Management

Water used for fish maintenance was aged overnight before the introduction of comet fish, which were then acclimatized for 24 h. Water changes, involving a 50% siphon and replacement with fresh water, were performed every 7 days (Riantono et al., 2016; Choeronawati et al., 2019). Water temperature and pH were measured daily (in the morning), whereas dissolved oxygen (DO) levels were recorded at the beginning and end of the maintenance period by Horiba U-50 (Water Quality Checker).

2.12. Data analysis

The parameters assessed in this study included clinical symptoms, absolute weight change, absolute length change, comet fish survival rate, *Argulus* behavior, *Argulus* detachment, and water quality. The effect of different papaya leaf extract concentrations on each parameter was statistically analyzed using one-way ANOVA to identify any significant differences between treatment groups, with a subsequent Duncan's Multiple Range Test for post-hoc analysis.

3. Results and Discussions

3.1. Results

3.1.1. Preliminary Test

Argulus is notably resilient compared with many other parasites, making it challenging to remove from infected fish. The assessment was carried out without the presence of the fish and evaluate the extract's direct influence on parasite behavior. The variation in behavioral stages across treatments provides a visual representation of how increasing concentrations influence parasite movement, agitation, and eventual immobilization. The results of observations of *Argulus* behavior can be seen in Table 1.

Table 1. Time-Dependent Behavioral Responses of *Argulus* to Different Concentrations of Papaya (*Carica papaya*) Leaf Extract

Time (min)	Treatment			
	0 ml L^{-1}	7 ml L^{-1}	14 ml L^{-1}	21 ml L^{-1}
10	a	a	a	a
20	a	a	b	b
30	a	a	b	c
40	a	a	b	c
50	a	b	b	c
60	a	b	c	c

Notes:

- **Behavior scale:**
  - *a* = no abnormal movement (actively/normal swimming)
  - *b* = mild behavioral changes (reduced swimming up and down)
  - *c* = moderate stress or reduced mobility (Weak swimming up and down)
  - *d* = severe immobilization (not moving/mortality)

Table 2. Qualitative phytocemical testing of papaya leaf extract

Compound	Result
Tannins	+
Alkaloids	+
Saponins	+
Flavonoids	-

Based on the observations presented in Table 1, all *Argulus* specimens initially exhibited active swimming upon immersion. The results, illustrates the progression of *Argulus* behavior over a 60-minute exposure period to different concentrations of papaya leaf extract (0, 7, 14, and 21 ml L^{-1}). At 0 ml L^{-1} ml L^{-1} (control), the parasites showed no behavioral change, remaining at baseline (level a-actively swimming) throughout the entire observation. At 7 ml L^{-1}, behavioral changes only began at 50 minutes, rising modestly to level b by 60 minutes. In contrast, at 14 ml L^{-1}, behavioral alterations appeared earlier, starting at 20 minutes and progressing gradually from actively swimming to the weakest condition by 60 minutes. The strongest and earliest responses were observed at 21 ml L^{-1}, where *Argulus* behavior increased rapidly from actively swimming to slower condition by 20 minutes and reached the weakest one by 30 minutes, maintaining this heightened state until the end of the experiment.

3.1.2. Phytochemical Test of Papaya Leaf Extract

Phytochemical screening of the papaya leaf extract demonstrated the presence of several bioactive compounds, specifically tannins, alkaloids, and saponins. These phytochemicals are known for their diverse biological activities, including antioxidant, antimicrobial, and anti-inflammatory properties, which may contribute to the therapeutic potential of the extract. In contrast, flavonoids, another important group of plant secondary metabolites often associated with health benefits, were not detected in the extract. The detailed qualitative results of this screening are summarized in Table 2, providing a clear overview of the phytochemical composition of the papaya leaf extract.

Argulus Behavior and Clinical Symptoms

Observations were randomly conducted across 12 aquaria after introduction of *Argulus* to the comet fish population. Attachment to the fish occurred within 2.55–9.35 minutes. Representative parasite attachment sites on the host are shown in Figure 1 and Table 3. Argulosis infection causes tissue lesions and bleeding in the fish body. Changes in clinical symptoms indicate lessions to the fish body parts can be seen in Figure 3. As the dosage levels changed, notable variations in movement, interaction, and physiological stress indicators were observed, indicating that different concentrations of the extract elicited specific behavioral adaptations.

Initial exposure trials revealed that *Argulus* rapidly located and attached to comet fish, with attachment times ranging from 2.55 to 9.35 minutes across 12 independently monitored aquaria (Table X). The parasite exhibited marked site specificity, preferentially attaching to anatomically vulnerable regions such as the tail fin, cranial surface near the gills, and the head.

(A) (B) (C)

Figure 1. *Argulus* Attached to the Tail Fin (A), Body Surface Near the Gills (B) and Head (C)

Table 3. Site of *Argulus* Attachment and Time Required for Initial Host Contact

Parameter	Observation
Time to first attachment (range)	2.55–9.35 min
Primary attachment sites	Tail fin (A); Body surface near gills (B); Head region (C)
Site characteristics	Thin epidermis; high vascularization; reduced scale protection
Implication	Facilitates rapid anchoring and increases infection efficiency

These locations are characterized by thin epithelial layers, reduced scale protection, and high vascularization, all of which facilitate parasite anchoring and feeding. The consistency of attachment across multiple tanks underscores the parasite's strong host-seeking capability and reinforces its high transmission potential in aquaculture settings. Representative attachment sites are shown in Figure 2.

The figure shows a comet fish exhibiting a localized area of erythema and surface abrasion, highlighted by the red circle. This reddish lesion is consistent with site-specific irritation caused by *Argulus* attachment, where the parasite's feeding and anchoring structures mechanically disrupt the skin. The affected region appears inflamed, indicating epidermal erosion and mild hemorrhage, which are characteristic host responses to *Argulus* parasitism. This visual evidence supports the pathological impact of the parasite on the integumentary integrity of the infected fish.

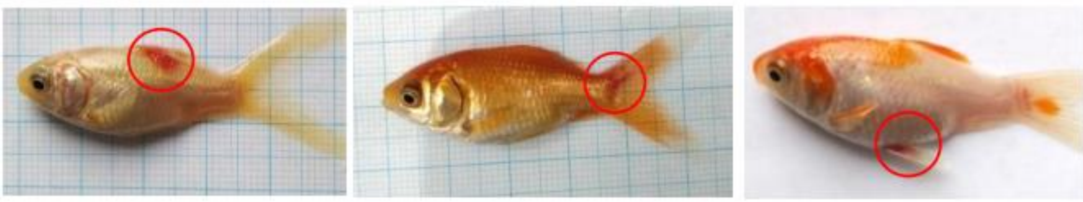
3.1.3. Survival Rate of Comet Fish

The survival rate of comet fish during exposure to papaya leaf extract is illustrated in Figure 3. It represents the survival values observed throughout the soaking period, reflecting the extract's effects on fish viability under the tested conditions. Monitoring survival rates is crucial to assess the safety and potential toxicity of the extract when applied to aquatic organisms. It also provides insight into how different concentrations or durations of exposure to the papaya leaf extract influence the overall health and survivability of the comet fish, serving as a foundational assessment for its suitability in therapeutic or experimental applications.

The survival rates of comet fish following soaking in the papaya leaf extract are shown in Figure 3. Treatments B (7 ml L^{-1}), C (14 ml L^{-1}), and D (21 ml L^{-1}) resulted in a 100% survival rate. In contrast, the control group (treatment A, 0 ml L^{-1}) had a survival rate of 86.67%. Statistical analysis using ANOVA revealed that the treatments did not significantly affect the survival of comet fish (F-statistic < F-critical at $p=0.05$), thus precluding the need for a post-hoc Duncan's test. However, the study's findings on *Argulus* detachment from comet fish indicated the positive effects of the treatments.

3.1. *Argulus* Detachment

The results obtained are based on the calculation of parasites detachment from 10 attached ones. This condition is recorded as the detachment of *Argulus* which can be seen in Figure 4. The observed *Argulus* detachment rates were as follows: treatment D (21 ml L^{-1}) yielded 70%, treatment C (14 ml L^{-1}) 33.33%, treatment B (7 ml L^{-1}) 16.67%, and the control (treatment A, 0 ml L^{-1}) 0%. *Argulus* death was not recorded during the soaking period, and 100% detachment was not achieved within this time, although it occurred later.



(A) (B) (C)

Figure 2. Comet fish exhibited localized tissue damage around the dorsal (A), caudal (B), and pelvic (C) fins as a result of *Argulus* infestation.

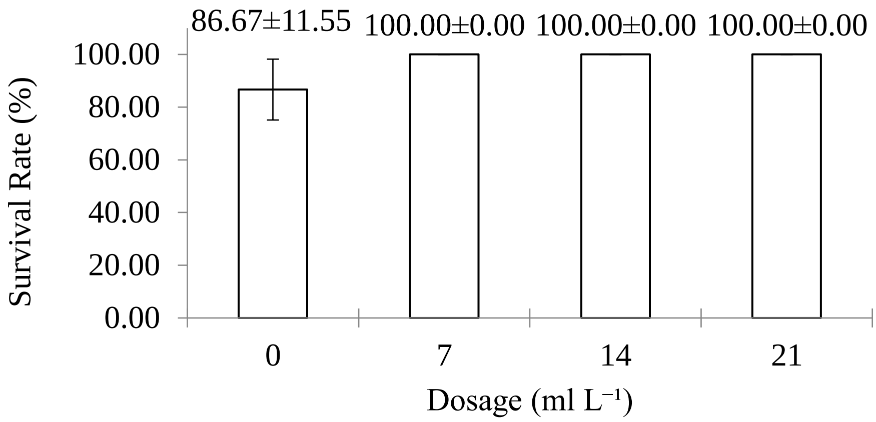


Figure 3. Survival Value Graph of Comet Fish When Soaked in Papaya Leaf Extract. Treatments with the same superscript indicate no significant difference (P>0.05)

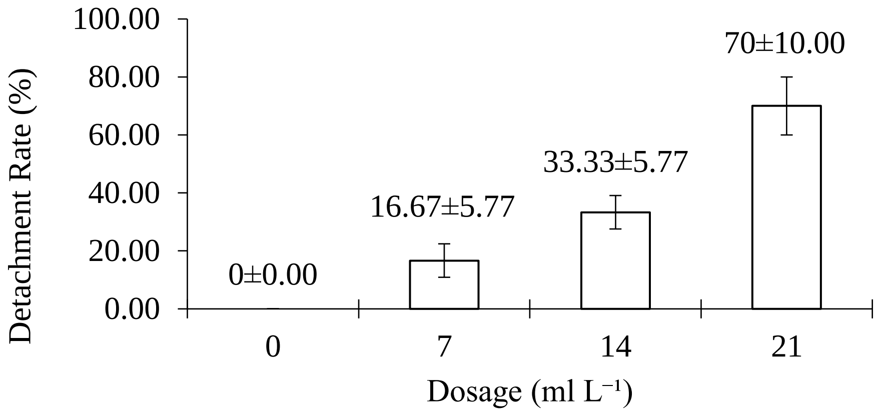


Figure 4. Detachment Rate Graph *Argulus* Post Papaya Leaf Extract Soaking. Treatments with different superscripts indicate significant differences (P<0.05)

3.2. Water Quality

Water quality was monitored throughout the 30-day study, revealing consistent parameters: temperature ranged from 23.6 to 24.5°C, dissolved oxygen (DO) levels were between 7.69 and 8.51 mg/L, and the pH fluctuated between 6.8 and 7.7. To maintain water quality, a 50% water change was performed every seven days via siphoning and replacement with clean water. Regular water changes are known to increase DO levels, thereby supporting the overall health and growth of the goldfish.

4. Discussion

Overall, the results demonstrate a clear dose- and time-dependent effect of papaya leaf extract on *Argulus* behavior, supporting its potential role in reducing parasite activity and attachment capacity. Understanding these patterns is crucial for interpreting the impact of the extract on Argulus and provides valuable insights into their behavioral ecology under papaya leaf extract exposure. Clinical manifestations such as excessive mucus secretion, tissue lesions, and behavioral impairment are characteristic responses of fish to *Argulus* infestation and reflect epithelial damage and physiological stress (Tavares-Dias & Martins, 2020; Rasheed et al., 2022). Reducing parasite attachment and mobility is a critical mechanism for alleviating clinical symptoms and limiting transmission in aquaculture systems (Rahman et al., 2021). Plant-derived extracts have gained attention as environmentally friendly antiparasitic agents that effectively reduce ectoparasite burden while supporting host recovery (Reverter et al., 2021; Dawood et al., 2022).

According to Pietoyo et al. (2022), a survival rate (SR) > 50% is considered good. During the *Argulus* infection phase, two comet fish died. Shameena et al. (2021) note that *Argulus* infestations can lead to secondary infections, subsequently causing fish mortality and reduced survival. Given the 100% survival rates in treatments B, C, and D, it is evident that papaya leaf extract not only mitigates *Argulus* infestation but also supports the overall health and viability of comet fish. Consistent with Harahap et al. (2021), a higher dose of papaya leaf extract appeared to accelerate *Argulus* release without affecting fish survival rate. This could be a suggestion for further research to study greater doses of papaya leaf extract to obtain a significant result on the survival rate of the comet fish.

Harahap et al. (2021) defined 100% detachment as an indication of high treatment efficacy. The release of parasites is thought to be a consequence of papaya leaf extract altering the environment, which disrupts the swimming capabilities of *Argulus*, making it difficult for them to remain attached to fish (Harahap et al., 2021). It is suspected that increasing the concentration of papaya leaf extract might be necessary to induce parasite mortality. Sari et al. (2018) have shown that higher extract doses lead to a faster rate of *Argulus* detachment. The ANOVA results indicated a significant impact of the treatments on *Argulus* detachment (F-statistic > F-critical, $p<0.05$), leading to the use of Duncan's test for further comparison. This suggests that papaya leaf extract is indeed effective in facilitating the release of these parasites, likely due to the presence of the antiparasitic alkaloid carpaine (Harahap et al., 2021).

The effectiveness of papaya leaf extract administration has an effect on the condition of *Argulus*. This effect is characterized by the movement of parasite swimming activity, which is disrupted by the addition of papaya leaf extract until it is released from the host body (Harahap et al*.,* 2021). The parasitic nature, which tends to be temporary, shows a free-swim pattern of finding a host and moving the target of attachment (Harlina et al*.,* 2019). The swimming pattern leads to a flushing movement (up and down), swimming around the target of attachment or towards the fish. Excessive mucus production can indicate parasite infection, where the fins and tail of the fish are the outer parts of the body that are easily infected (Nurani et al*.,* 2020). There are wounds on the dorsal fin, pectoral fin, pelvic fin, caudal fin, stomach, head and body surface in certain parts. This parasite pierces the host's skin to suck the host's blood which will cause lesions on the surface of the skin (Thakur et al*.,* 2023). Furthermore, the presence of red spots on the body surface, swelling around the gills, and fins damage shows the infection by *Argulus*, which occur due to anticoagulant enzymes released by these parasites when they stung with a stylet (Noga, 2010; Stoskopf, 1993).

A dose-dependent effect of the papaya leaf extract, potentially due to its alkaloid content. Sari et al. (2021) propose that higher alkaloid concentrations lead to increased parasite weakness and passivity. The observed disruption of *Argulus* movement indicates that the papaya leaf extract affects the physiological state of the parasite. The rapid weakening in treatment D (21 ml L^{-1}) further supports this, likely due to the presence of the alkaloid carpaine in the extract, which Azizah et al. (2019) note has a direct antiparasitic effect. This detachment is due to the antiparasitic properties of alkaloids present in papaya leaves, such as carpaine, pseudocarpaine, dehydrocarpaine I, dehydrocarpaine II, carposide, and emetine. Moreover, papaya leaf extract possesses antioxidant substances that enhance the immune system of goldfish by initiating superoxide dismutase (SOD) and catalase (CAT) in goldfish with *Argulus* infection. This causes reactive oxygen species (ROS) generation and cellular damage in the *Argulus*, leading to the parasite detachment (Boopathi et al., 2024; Yap et al., 2021).

Phytochemical screening revealed the presence of tannins, alkaloids, and saponins in the papaya leaf extract, while flavonoids were not detected. While the flavonoid test resulted in a brownish coloration, a positive result for flavonoids, according to Tutik et al. (2022), was indicated by a color change to orange-red or purple-red. Saponins have role in controlling pathogens and parasites and enhancing immune responses in fish (Ekanem et al., 2004; Vuong et al., 2014). Similar to saponins, alkaloids and tannins contribute to anti-parasitic and antioxidant activities (Singh et al., 2020). The combination of these bioactive constituents—particularly tannins and alkaloids, which are associated with antiparasitic activity—suggests that the extract possesses compounds capable of contributing to *Argulus* control.

These measurements fall within the recommended ranges for goldfish (*Carassius auratus*) culture, specifically a DO level above 4 mg/L and a pH between 6.5 and 8.5 (SNI 8110, 2015), as well as a temperature range of 20-30°C (Agustina et al., 2018). Additionally, water environments with DO levels higher than 5 mg/L mg/L can inhibit *Argulus* development (Austin & Austin, 2016). Thus, it prevents the *Argulus* infestation in goldfish. Brett (1979) stated that when DO level decreases, it will lead to the lower capability to respirate and feed of the fish. As a result of hypoxia, especially during prolonged exposure, metabolic and physiological activities are also affected (Wedemeyer, 1996).

From an epidemiological standpoint, the patterns observed in this study reflect clear disruptions to key processes that drive *Argulus* transmission. Exposure to papaya (*Carica papaya*) leaf extract markedly reduced parasite burden and impaired essential behaviors—such as locomotion, host-seeking activity, and attachment capability—which collectively diminish the parasite's effective contact rate with hosts, a core determinant of transmission potential (Ogut et al., 2005). Such behavioral impairment likely reduces the parasite's basic reproduction potential, thereby lowering infection pressure within the system. The dose-dependent response further supports epidemiological principles indicating that greater intervention intensity yields stronger suppression of pathogen persistence. Higher extract concentrations produced more rapid and pronounced functional deficits in *Argulus*, consistent with evidence that reduced parasite viability can substantially limit outbreak propagation in aquaculture environments (Bandilla et al., 2006).

Improvements in fish condition and reduced lesion severity following treatment also suggest enhanced host resilience—an essential component of the host–pathogen–environment triad. By limiting tissue compromise and potential entry points for secondary pathogens, the extract may indirectly decrease the risk of co-infections and improve overall population health. Collectively, these findings indicate that papaya leaf extract functions not only as a therapeutic agent but also as an epidemiologically relevant intervention capable of lowering infestation pressure and constraining parasite transmission dynamics at the population level.

5. Conclusion

This study demonstrates that immersion treatment using papaya (*C. papaya*) leaf extract at varying doses is effective in controlling *Argulus* infestation in comet fish (*C. auratus*). The treatment significantly improved the clinical condition of infected fish, as evidenced by the restoration of normal behavior, while maintaining a 100% survival rate, indicating good tolerance and absence of acute toxicity. Statistical analysis confirmed that the extract had no adverse effect on fish survival ($P > 0.05$). Moreover, papaya leaf extract promoted parasite detachment in a dose-dependent manner, with the highest efficacy observed at 21 ml L^{-1}, resulting in 70.00 ± 10.00% parasite release from the host. These findings support the potential of papaya leaf extract as a safe and effective phytotherapeutic agent for managing *Argulus* infestation in ornamental fish culture.

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