

**Research Article**

Effectiveness of Papaya Leaf Extract Immersion in Controlling *Argulus* Infestation on Comet Fish (*Carassius auratus*)

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Abstract

Influenza outbreaks in aquaculture, coupled with the growing resistance to chemical antiparasitics, underscore the urgent need for safer botanical alternatives. Infestations for *Argulus* spp. lead to substantial stress and mortality in ornamental fish, yet effective and low-risk treatments remain scarce. Therefore, this study evaluated the effectiveness of papaya (*Carica papaya*) leaf extract at different immersion durations in controlling *Argulus* infestation in comet fish (*Carassius auratus*). Sixty fish (4.94 ± 0.28 cm; 1.32 ± 0.22 g) were randomly distributed into 12 aquaria and infected with 120 *Argulus* spp. parasites. The experiment followed a completely randomized design with four immersion durations, 0, 45, 60, and 75 minutes, each replicated three times, using papaya leaf extract prepared by infusion at 14 mL/L. Immersion in the extract did not significantly affect fish survival, which remained high across treatments ($93.33 \pm 11.55\%$ in the control and $100.00 \pm 0.00\%$ in all treated groups). However, *Argulus* detachment increased with immersion time, reaching $60.00 \pm 10.00\%$ at 75 minutes. Exposed parasites initially showed vigorous movement that weakened over time, eventually leading to detachment and immobility. These results indicate that a 75-minute immersion in 14 mL/L papaya leaf extract effectively induces parasite detachment and alleviates clinical symptoms without compromising fish survival, supporting its potential as a safe botanical antiparasitic for ornamental fish culture.

Keywords: *Argulus*, *Carassius auratus*, detachment, immersion time, papaya leaf extract

1. Introduction

The comet fish (*Carassius auratus*) is one of the most popular ornamental fish species and has strong potential as a valuable aquaculture commodity in Indonesia for both domestic and international markets [1]. Despite its economic potential, comet fish farming, like other ornamental aquaculture systems, is vulnerable to infectious diseases, which represent one of the greatest challenges to sustainable production [2].

In fish disease epidemiology, the emergence of disease is understood to be the result of interactions among the host, pathogen, and environment [3]. Changes in environmental quality, high stocking densities, and poor biosecurity practices

increase disease transmission and outbreaks in aquaculture systems [4, 5]. Among parasitic diseases, *Argulus* infestation is one of the most common and detrimental. This ectoparasite can be introduced into ponds through aquatic plants, contaminated equipment, and animal carriers [6]. Once infested, fish may experience tissue damage, bleeding, impaired swimming, imbalance, and excessive mucus secretion [2].

The spread of *Argulus* in aquaculture reflects key epidemiological patterns, including rapid transmission under favorable conditions, direct effects on host health, and indirect economic losses due to reduced fish survival and market value.

Traditionally, farmers have relied on chemical control, such as dimilin, but this method is costly and can negatively affect aquatic ecosystems when overused [7].

In line with epidemiological approaches that emphasize prevention, sustainable control, and reduction of pathogen prevalence, herbal-based alternatives are being increasingly considered. Papaya (*Carica papaya*) leaf extract contains bioactive compounds such as carpain, which prevents parasite attachment, and saponins, which disrupt parasite metabolism, leading to parasite mortality [8]. These natural compounds offer a promising solution for reducing the parasite burden while minimizing environmental risks. [9] reported that a 30% concentration of papaya leaf juice was able to detach approximately 88% of *Argulus* from comet fish within 20 minutes, while [10] identified the alkaloid carpain in papaya as a compound with strong *Argulus*-detaching activity in. Furthermore this study employs a remarkably lower concentration (14 mL/L or 1.4%) from prior studies [8] as the optimal dose and shifts the focus from dosage levels to immersion duration as the primary factor influencing *Argulus* removal.

2. Material and Methods

The study was conducted from February 19 to March 18, 2024, at the Kebowan Fish Seed Center in Central Java. Fish were selected based on uniform size, body weight, physical condition, active feeding response, and normal swimming behavior. A total of sixty comet fish were stocked into 12 aquaria at a density of one fish per liter, following the method of [2]. The fish had an average total length of 4.83 ± 0.20 cm and an average body weight of 1.30 ± 0.12 g. Prior to experimentation, the fish underwent a 24-hour acclimation period without feeding to allow physiological adaptation [11, 12]. This was followed by a seven-day rearing period to ensure that all individuals were healthy and free from disease, confirming their suitability for use in the study. During this period, the fish were fed to apparent satiation twice daily, in the morning and evening. *Argulus* parasites were collected from ornamental fish vendors at Pasar Raya II in Salatiga. A total of 120 *Argulus* were used, with 10 parasites introduced

into each aquarium.

The study employed an experimental approach using a Completely Randomized Design (CRD) with four treatments and three replicates. The treatments consisted of Treatment A (0 min), Treatment B (45 min), Treatment C (60 min), and Treatment D (75 min), each applied at a uniform concentration of 14 mL/L.

2.1. Extract preparation

Papaya leaves were used at a concentration of 100 g/L, with mature leaves selected for extraction. The extract was prepared using the infusion method described by [13], involving boiling the leaves for 15 minutes at 90°C. Leaf color selection was conducted using the RGB (Red 5, Green 69, Blue 17) method. The local “California” papaya leaves were sourced from the Kebowan Fish Breeding Center (BBI Kebowan). Additionally, mature papaya leaves are known to contain high levels of phenolic compounds [14], with alkaloid content reported at 4.38% per 5 g of papaya leaves [15].

2.2. Experimental procedure

Comet fish (*C. auratus*) were acclimated for seven days before the experiment. Each treatment tanks were artificially infected with 10 *Argulus* parasites. The infection process lasted for four days to allow the development of clinical symptoms. Following infection, fish were immersed in papaya leaf extract (14 mL/L) for the designated treatment durations of 45, 60, and 75 minutes. The selected concentration was determined based on the findings of [8], who tested doses of 0, 7, 14, and 21 mL/L and identified 14 mL/L as the optimal dose.

2.3. Observation parameters

Observations were conducted for seven days after immersion. The parameters assessed included the clinical symptoms of the host, behavioral responses of *Argulus*, fish survival rate, parasite detachment, and water quality.

2.4. Phytochemical analysis

The phytochemical screening of the different *C. papaya* obtained extracts was carried out to ensure the presence of certain chemical families; it was determined by solubility tests, color reactions by characteristic reagents and precipitation. The phytochemical tests conducted included tests for alkaloids,

tannins, flavonoids, and saponins. The alkaloids were highlighted by the reagents of Mayer, Dragendorff [16, 17], the tannins by ferric chloride [18, 19], saponins were determined based on their foam-forming abilities [20], polyphenolic substances by FeCl_3 [21], and the revelation of flavonoids by the reaction with cyanidine [22].

2.5. Clinical symptoms

Clinical symptoms were recorded through visual observation before and during infection with *Argulus* and after immersion in papaya leaf extract. The parameters observed included feeding response, swimming activity, and external morphological changes in the experimental fish.

2.6. *Argulus* behavior

Behavioral observations of *Argulus* were conducted during the infection period of the fish. Unlike many other parasites, *Argulus* exhibits high resistance and is difficult to eliminate from fish. Observations focused on swimming patterns, including the approach of *Argulus* toward the host, time required to reach the host, and movement patterns during flushing (uncontrolled vertical movements). The site preference of parasite attachment on the body surface of comet fish was also documented.

2.7. 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 [23]:

$$\text{SR} = \text{Nt}/\text{N0} \times 100\%$$

2.8. 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 Puspitasari [9].

$$P = a/b \times 100\%$$

2.9. Water Quality

The water quality parameters measured included temperature, pH, and dissolved oxygen (DO). Temperature and pH were recorded daily, and DO was measured at the beginning and end of the maintenance period.

2.10. Data analysis

Survival rate (SR) and mortality rate (MR) were analyzed using SPSS 16.0 (IBM Statistics) with Duncan's multiple range test, and results are presented as mean \pm SE. Clinical symptoms and water quality parameters were assessed descriptively.

3. Results

The observations were conducted in the absence of fish to specifically assess the direct effect of the extract on parasite behavior. Based on the results of the *in vitro* test, immersion in papaya leaf extract at a concentration of 14 mL/L affected the condition of *Argulus*, causing reduced movement and increased passivity. At the start of immersion, *Argulus* in all treatments exhibited active flushing movements. A noticeable decline in activity began at minute 25 in Treatment B and around minute 30 in Treatments C and D, followed by pronounced weakening at minute 60 in Treatments C and D (Table 1).

Table 1. *Argulus* behaviour during treatments with the absences of fish

Time (Minutes)	Treatment			
	A(Control)	B (45 mins)	C (60 mins)	D (75 mins)
5	+++	+++	+++	+++
10	+++	+++	+++	+++
15	+++	+++	+++	+++
20	+++	+++	+++	+++
25	+++	++	+++	+++
30	+++	++	++	++
35	+++	++	++	++
40	+++	++	++	++
45	+++	++	++	++
50	+++		++	++
55	+++		++	++
60	+++		+	+
65	+++			+
70	+++			+
75	+++			+

Note: : (+ = Weak; ++ = Reduced; +++ = Active).

Table 2. Phytochemical testing of papaya leaf extract using the infusion method

Compound Name	Results
Saponins	+
Flavonoids	-
Alkaloids	+
Tannins	+

Note: ((+ = Positive Result (Contains the tested compound); - = Negative Result (Does not contain the tested compound)).

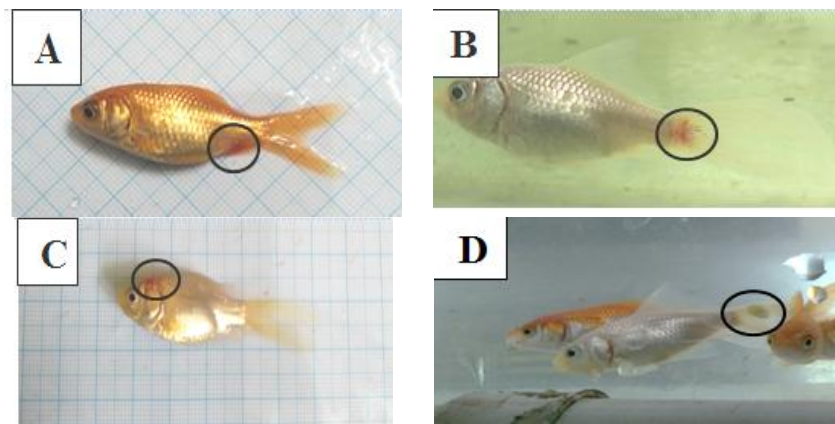


Figure 1. Clinical Symptoms After *Argulus* Infestation.

Table 3. Observation of *Argulus* behavior on fish during immersion treatment.

Time (min)	Swimming Pattern	Attachment Time	Observation Notes
2–10	Active, fast movement (+++)	Strong attachment	Highly mobile parasites
20–30	Reduced activity (++)	Moderate	Some detachment observed
45–60	Weak movement (+)	Weak	Many detached parasites
75	Inactive (– / +)	Very weak	Almost all detached

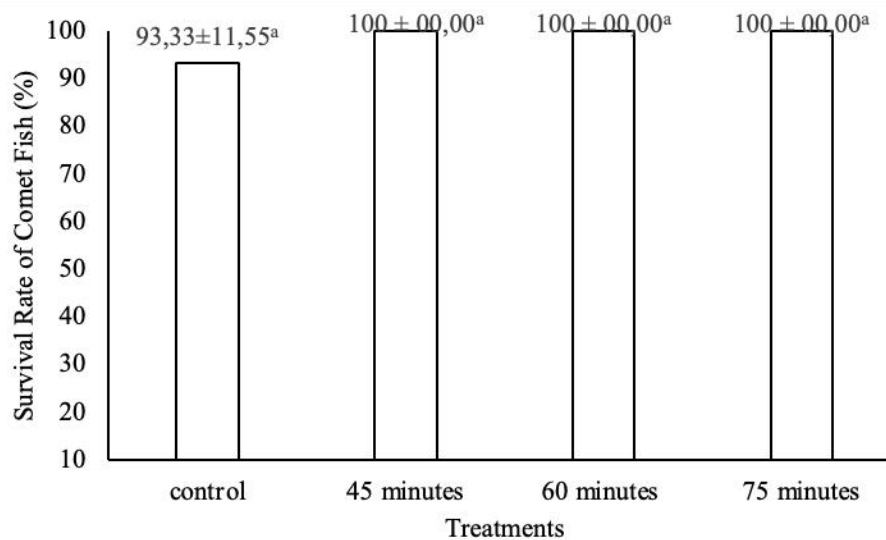


Figure 2. Survival rate of comet fish after treatments.

The activity of *Argulus* decreased progressively with increasing immersion time in papaya leaf extract (Table 3). During the initial 5–20 minutes, *Argulus* in all treatments remained highly active (“+++”), indicating strong attachment to the host. Activity reduction began after 25 minutes in the

immersion treatments (B, C, and D), while the control group (A/Control, 0 min) maintained consistent activity throughout. By 45–60 minutes, *Argulus* activity in Treatments C and D declined markedly (“++” to “+”), showing reduced movement and attachment strength. At 75 minutes, most *Argulus* in Treatment D showed weak or no activity, whereas those in the

control remained active. These findings indicate that longer immersion times effectively weakened and detached then killed *Argulus*, suggesting a strong time-dependent antiparasitic effect of papaya leaf extract.

The results showed that the papaya leaf extract obtained using the infusion method contained the active compounds saponins, alkaloids, and tannins but did not contain flavonoids.

Clinical observations of comet fish were conducted before infection, during *Argulus* infection, and after papaya leaf extract immersion, covering the behavioral and morphological conditions. The behavioral condition of fish before infection was normal swimming and feeding responses. During infection, the fish were inactive and had a reduced feeding response. After immersion in the extract, the fish swam normally and had a normal feeding response. Morphologically, the fish were healthy before infection, with no defects. After infection, the fish exhibited wounds, bleeding, and excessive mucus. After immersion in the extract, the wounds began to heal, there was no bleeding, and the wounds and mucus were no longer excessive. The clinical symptoms of comet fish after *Argulus* infection are shown in figure 2. The behaviour of *Argulus* was observed when *Argulus* was first placed in the research aquarium. The *Argulus* behavioural data are presented in table 3.

The survival rate of comet fish did not differ significantly among treatments ($p > 0.05$, one-way ANOVA), with treatments B (45 min), C (60 min), and D (75 min) showing the highest mean survival of $100.00 \pm 0.00\%$, while treatment A (0 min) had a slightly lower mean of $93.33 \pm 11.55\%$. These results indicate that immersion in papaya leaf extract, even at longer durations, did not adversely affect fish survival.

The results of the detachment of *Argulus* are shown in figure 3. The highest average percentage detachment of *Argulus* was observed in treatment D (75 minutes) at $60.00 \pm 10.00\%$, followed by treatment C (60 minutes) at $43.33 \pm 11.54\%$ and treatment B (45 minutes) at $30.00 \pm 10.00\%$. The lowest average percentage detachment of *Argulus* was observed in treatment A (0 min) at $0.00 \pm 0.00\%$. The detached parasites adhered to the walls of the container or aquarium and

exhibited no further movement, indicating mortality. This observation was confirmed at the end of the experiment, as the *Argulus* remained in the same position and detached only during manual cleaning of the container. The results of the water quality observations during the study are presented in table 4.

Table 4. Water quality parameters during the experimental period compared with standard values Notes: a) [24]; and b) [25]

Parameters	Observed Range	Standard Range
Temperature (°C)	23,1-25,2	^(a) 20-30
pH	6,8-7,8	^(b) 6,5-8,5
DO (mg/l)	7,20-8,95	^(b) > 5

The water quality parameters observed were temperature, pH, and DO levels. Temperature and pH measurements were taken daily in the morning and afternoon, while DO measurements were taken at the beginning and end of the maintenance period.

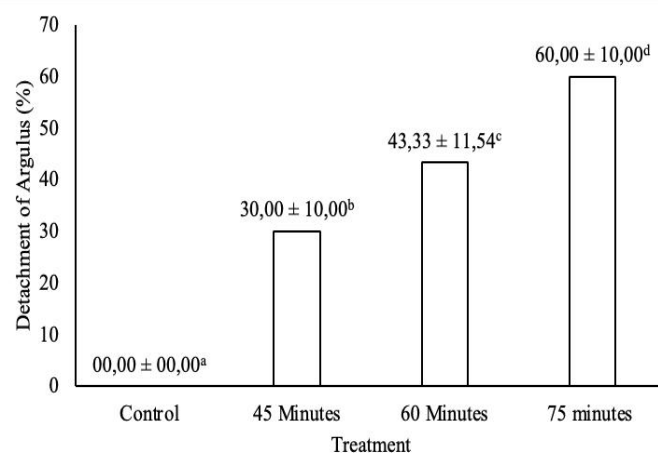


Figure 3. *Argulus* detachment after immersion in extract. Note: Treatments followed by different superscripts are significantly different.

4. Discussion

Argulus performed flushing or uncontrolled up-and-down movements when placed in the extract. Exposure to the extract initially triggered vigorous flushing movements in *Argulus*, indicating irritation or stress, which was subsequently followed by reduced activity and passivity. Based on this response, an initial hypothesis was formulated that *Argulus*

attached to fish could be detached with the appropriate dose and duration of immersion. This aligns with [8], who found that immersion in papaya leaf extract makes *Argulus* uncomfortable owing to environmental changes, preventing it from adapting. The results indicate that the longer the immersion duration, weaker the *Argulus* movements.

Phytochemical testing was performed to identify the active compounds in papaya leaf extract. Based on the testing, it can be concluded that the papaya leaf extract prepared using the infusion method contains the active compounds saponin, alkaloids, and tannins. No flavonoids were detected in the extract. Although several studies have reported the presence of flavonoids in papaya (*C. papaya*) leaves [26, 20], the present study did not detect these compounds in the infusion extract. This discrepancy may be attributed to several methodological factors. First, the extraction method used in this study employed a hot-water infusion at 90 °C, which may have been less effective for extracting semi-polar compounds such as flavonoids and could have led to thermal degradation of heat-sensitive components [15]. Previous research has shown that ethanol or methanol solvents yield higher flavonoid concentrations than water extractions [26]. Second, the maturity and variety of leaves can influence phytochemical content; mature leaves generally contain higher levels of flavonoids than young leaves [27]. Lastly, the qualitative phytochemical tests used in this study may not have been sensitive enough to detect low concentrations of flavonoids, particularly if their levels were near the detection limit. Therefore, future studies should consider employing ethanol-based maceration or quantitative spectrophotometric assays to verify the presence of flavonoids in papaya leaf extracts. Saponins and Glycosides are known for their potential role in disrupting parasite cell membranes. Papaya extracts contain these compounds, which can contribute to their antiparasitic activity [28]. On the other hand, alkaloids are known for their wide range of biological activities, including antibacterial and antiparasitic properties. These compounds are present in papaya leaf extracts and contribute to their bioactive potential [29, 30]. And also, tannins as a phenolic compound, present in

both the leaves and seeds of papaya, have antioxidative properties that can enhance the plant's defense mechanisms against pathogens, including parasites [31]. These bioactive compounds work synergistically, enhancing the antiparasitic capabilities of papaya extracts, making them a potential natural treatment option for managing *Argulus* infestations in aquaculture.

Clinical symptoms in the form of visible behaviours include swimming patterns and responses to food. These behavioural changes are thought to be due to comet fish experiencing stress from *Argulus* attacks and blood loss. According to [32], fish infected with *Argulus* experience stress due to wounds and significant blood loss. Clinical symptoms on the external body parts of comet fish include wounds on the body surface, bleeding on the dorsal, tail, and anal fins, and excessive mucus production. Wounds and bleeding in comet fish are caused by *Argulus* parasites attaching themselves to the fish, sucking their blood, releasing toxins, and causing irritation from their stylet. According to [8], *Argulus* infestation in comet fish causes wounds on the body surface due to the stylet, bleeding, irregular swimming, loss of balance, and excessive mucus production in the body. After soaking in papaya leaf extract, the clinical symptoms of the comet fish improved. The wound healing process is believed to be due to the presence of active compounds in the papaya leaf extract. According to [33], papaya leaves contain papain enzymes that are used as immunomodulators to enhance the immune system. According to [34], the wound healing process, such as fading wounds and bleeding, is due to the entry of antibacterial compounds such as alkaloids, which can damage cell walls, inhibit division, and boost the immune system of fish.

The observation of behavior is based on the nature of *Argulus*, which differs from that of other parasites in that *Argulus* is difficult to kill and can even feign death. Therefore, a more detailed observation of *Argulus* behaviour is necessary. Based on observations of swimming patterns, when *Argulus* is introduced into an aquarium, it first swims around and floats while occasionally approaching comet fish but does not immediately attach itself. Some *Argulus*, after swimming

around, attach themselves to the aquarium walls or remain at the bottom of the aquarium. *Argulus* begins to attach itself to comet fish after 2–10 minutes. The prolonged attachment of *Argulus* is likely due to the agile movements of the fish. This study acknowledges certain limitations, particularly in the assessment of *Argulus* behaviour. Observations of parasite movement were recorded qualitatively, without quantitative behavioural metrics such as the frequency or duration of flushing movements. As a result, the interpretation of behavioural responses remains descriptive rather than statistically measurable. Based on observations, *Argulus* attaches to the surface of the body, such as the body, head, near the gills, dorsal fin, anal fin, and tailfin. According to [35], *Argulus* spp. is commonly found on the outer parts of the body, such as the body surface, mucus, fins, and tail.

Based on the research, the survival rate of comet fish was as follows: $93.33 \pm 11.55\%$ in the treatment (0 min), $100.00 \pm 0.00\%$ in the treatment (45 min, 60 min, 75 min). Based on the results, it was found that papaya leaf extract at a dose of 14 ml/L and varying immersion times did not significantly affect the survival rate of comet fish infected with *Argulus* spp.. Papaya leaf extract did not cause mortality in the comet fish. This is consistent with [8], who stated that immersing *Argulus*-infected fish in papaya leaf extract at various doses and immersion times of 12 h did not cause fish mortality. This finding is consistent with [10], who similarly reported no fish mortality within one hour after treatment with papaya leaf extract.

The highest detachment rate of *Argulus* ($60.00 \pm 10.00\%$) was observed in treatment D (75 min), followed by $43.33 \pm 11.54\%$ in treatment C (60 min) and $30.00 \pm 10.00\%$ in treatment B (45 min), while no detachment occurred in treatment A (0 min). The detached *Argulus* were observed to be immobile and adhered to the aquarium walls until the end of the experiment, indicating that detachment was associated with parasite death rather than recovery. The detachment of *Argulus* may be due to the presence of papaya leaf extract containing the alkaloid compound carpain in the culture medium, which makes *Argulus* uncomfortable and causes it to detach from comet fish.

Alkaloids are toxic to arthropods, such as *Argulus*, causing paralysis and ultimately death [8]. According to [10], the use of papaya leaf extract is effective in controlling *Argulus* infestation because it contains compounds that are used as antibacterial and antiparasitic agents. Saponin is another active compound found in papaya leaf extract. Saponin can form foam, which easily disrupts the metabolism of parasites such as *Argulus*, ultimately leading to their death [8]. Other compounds in the form of tannins in papaya leaf extract affect parasite growth by damaging the cell membranes within the parasite's body, causing paralysis or muscle weakness [36]. The results of this study are inconsistent with those of [8], who reported that *Argulus* mortality within minutes of immersion in papaya leaf extract. This is likely due to differences in the extraction methods used for papaya leaves. In the study conducted by [8], the extract used was a concentrated extract obtained through maceration, which may have contained a higher concentration of active compounds.

In this study, papaya leaf extract was prepared using the infusion method, which involves heating the plant material in hot water for a short duration. This approach may extract fewer heat-sensitive or non-polar compounds compared to the maceration method used by [8], which typically employs ethanol or methanol solvents and longer extraction times. Such differences in solvent polarity and extraction duration can influence the concentration of saponins, alkaloids, and flavonoids, potentially explaining variations in antiparasitic efficacy between studies. Infusion tends to yield more polar compounds, while maceration can extract a broader range of bioactive substances with higher stability [20, 8]. Therefore, the lower intensity of *Argulus* detachment observed in this study compared to [8], may be due to the lower extraction efficiency of certain non-polar bioactive compounds under aqueous infusion conditions.

Based on the results of water quality measurements during the study, the temperature range for all treatments was 23.1–25.2 °C. This temperature range is suitable for maintaining comet fish. This is supported by [10], who stated that comet fish can grow well at temperatures of 20–30 °C. The pH range

during the study across all treatments was 6.8–7.8. This value is suitable for maintaining comet fish. This aligns with [25], which states that the optimal pH for maintaining comet fish is 6.5–8.5. DO measurements across all treatments in the study ranged from 7.20–8.95. These DO measurement results are suitable for maintaining comet fish. According to [25], the optimal DO for maintaining comet fish is >5 mg/l. The addition of papaya leaf extract during maintenance did not degrade water quality. The water quality remained suitable for comet fish maintenance. This aligns with [8], who stated that the water quality in cultivation media treated with papaya leaf extract immersion remains suitable for comet fish maintenance. Water quality parameters, including temperature, pH, and dissolved oxygen, remained within the optimal ranges for comet fish during the experiment. However, these parameters were not statistically analyzed (e.g., using ANOVA) to evaluate potential differences among treatments, which may limit the interpretation of treatment effects on water quality stability.

The use of the infusion extraction method to control *Argulus* caused *Argulus* to detach from comet fish and weakened its movement. Based on the results of *Argulus* detachment, the highest detachment rate was 60.00 ± 10.00 (Treatment D). No mortality was observed in *Argulus* during immersion in the extract using this method. This differs from the study conducted by [8], where immersion in papaya leaf extract using the maceration extraction method resulted in *Argulus* mortality of $80.00\% \pm 0.00$ at a dose of 14 ml/L. The extraction method used should be considered. The infusion extraction method uses simple equipment, resulting in low operational costs and environmental impact. Preparing the extract via infusion is easy, inexpensive, and quick, thereby saving costs and labor. This contrasts with the maceration extraction method, which uses numerous expensive tools, involves a difficult preparation process, and takes a long time [37]. Based on this comparison, extraction using the infusion method is more suitable for fish farmers in terms of cost and process complexity. Although it does not cause mortality in *Argulus*, alternative treatments can be applied, such as

quarantining the fish after all *Argulus* have been removed, cleaning the rearing containers, draining the water onto the ground, and replacing it with freshwater.

5. Conclusion

The present study demonstrated that immersion of comet fish in papaya leaf extract (14 mL/L) for 75 minutes was the most effective treatment, achieving up to 60% *Argulus* detachment, weakened movement, the finally caused *Argulus* mortality without adversely affecting fish survival or water quality,

However, since complete parasite elimination was not achieved, the efficacy of papaya leaf extract should be considered partial under the tested conditions. Future studies are recommended to explore higher concentrations, longer exposure times, or combination treatments with other natural antiparasitic agents to enhance control effectiveness and achieve full *Argulus* eradication.

Author contributions

A.H.C.H. , R.N.A., and D. designed the study. A.H.C.H and R.N.A. wrote the manuscript and performed the data analysis and curation. A.H.C.H and D revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethical approval

All research procedures involving fish were performed in accordance with the ethical principles for animal research as outlined in the Council for International Organizations of Medical Sciences (CIOMS) Guidelines, 2016. The study complied with both institutional and international standards for the ethical conduct of research involving animals.

Conflicts of Interest

The authors report no conflicts of interest.

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Data availability statement

The data presented in this study are available on request from the corresponding author.

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