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ORIGINAL ARTICLE

Protective effect of *Terminalia catappa* leaf extracts against Saprolegniosis on angelfish eggs

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Abstract

The Saprolegniosis causes major reduction egg hatching rate by angelfish (*Pterophyllum scalare*); therefore, we investigated the effectiveness of two *Terminalia catappa* extracts (27 °C and 80 °C) against *S. parasitica* on fish eggs. Mycelium growth was evaluated in vitro at 0, 2.5, 5.0 and 10 g/L for 96 h, in solid and liquid medium culture. In an in vivo assay, we tested lower concentrations of hot extract (25, 50 and 100 mg/L) to prevent infection by zoospores. The hot aqueous extract was the most effective, and the concentrations of 5 and 10 g/L demonstrated great efficiency in liquid medium. In vivo conditions, 50 and 100 mg/L of hot extract provided more viable eggs, greater percentage of total larvae and lower percentage of infected eggs. Thus, the hot extract should be used instead of the room-temperature extract as a prophylactic measure, to prevent saprolegniosis in *P. scalare* eggs and improving the hatching rate.

KEYWORDS
angelfish, effectiveness, Indian almond extract, phytotherapies, *Saprolegnia parasitica*

1 | INTRODUCTION

The oomycete *Saprolegnia* sp. infects freshwater fish, resulting in mortality and economic loss in fish farms (Shaalan et al., 2018). This pathogen affects fish in all development stages, from eggs to adults. It damages the chorionic membrane of eggs, quickly infecting all spawning and killing viable eggs (Liu et al., 2014). *Pterophyllum scalare* is among the fish species that are sensitive to this disease (Eissa et al., 2013).

Currently, few chemical products are legal to control fungal disease in Brazilian aquaculture, although some chemotherapy drugs have been used such as malachite green, iodopovidone, formalin,

copper sulphate, potassium permanganate, sodium chloride, hydrogen peroxide, acetic acid, iodine and methylene blue (Fuangsawat et al., 2011). Many of these products exhibit carcinogenic and toxicological effects in humans and animals (Phillips et al., 2008).

Phytotherapy stands out as an alternative treatment for disease outbreaks in fish farming due to its lower cost, less environmental impact and greater availability compared with chemicals or commercial products (Saha et al., 2017).

Various plant extracts have been used to control *Saprolegnia parasitica* on fish eggs, such as *Opuntia stricta* ethanolic extract on *Sander lucioperca* eggs (57.8% inhibition) (Khemis et al., 2016), *Kaempferia galanga* rhizome extract on *Clarias gariepinus* eggs (approximately 80% inhibition) (Humsari & Junianto, 2017); *Matricaria chamomilla* extract on *Oncorhynchus mykiss* eggs (hatching rate 75.90%) (Amiri & Meshkini, 2019); and garlic skin aqueous-methanolic extract or garlic stem aqueous-methanolic extract on rainbow trout eggs, which promoted a higher number of larvae (Özçelik et al., 2020).

The plant *Terminalia catappa* is commonly called Indian almond. Its biological activity is due to active compounds, such as flavonoids, terpenoids and tannins, which provide antimicrobial (Silva et al., 2015), antioxidant (Venkatalakshmi et al., 2015), anti-inflammatory (Abiodun et al., 2016), antiparasitic (Claudiano et al., 2009), antidiabetic (Hayaza et al., 2019) and antimutagenic activity (Mininel et al., 2014).

T. catappa extract has been used to promote fish health (Lusiastuti et al., 2017), to reduce fish stress during transportation (Sung, & Abol-Munafi, 2019), to promote breeding of blackwater fish species (Sung & Abol-Munafi, 2019), to assist in the health of *Betta splendens* after agonistic encounters (Chitmanat et al., 2005), to improve breeding activity (Lee et al., 2016), to increase survival and growth performance (Nurhidayat et al., 2016), to increase hatching of fish eggs and to promote growth of live food such as *Paramecium* (Sung & Abol-Munafi, 2019). However, there are no reports about the effects of aqueous *T. catappa* extract extracted with high temperature to control *Saprolegnia* sp. in *Pterophyllum scalare* eggs.

P. scalare (freshwater angelfish) is an important species in the ornamental fish market and intensively bred around the world; however, infection by *Saprolegnia parasitica* leads to low egg survival (Chambel et al., 2014).

Therefore, this study aimed to evaluate the effectiveness of *T. catappa* leaf aqueous extract to inhibit the growth of *S. parasitica* on *P. scalare* eggs.

2 | MATERIAL AND METHODS

2.1 | Plant extract production

Aqueous extracts of *Terminalia catappa* leaves were prepared employing the method of Meneses et al. (2020). Fallen leaves were washed with distilled water and dried in a laboratorial oven (50 °C) until constant weight. Afterwards, the whole sample was ground using a cutting mill (Marconi®). The aqueous extracts were made using 25 g of *T. catappa* leaves, soaked in sterile distilled water (500 mL in a glass beaker) for 1 h at two different temperatures (hot temperature of 80 °C and room temperature of 27 °C). The solutions were then filtered, and the retaining material was subjected to a re-extraction procedure (two-step extraction employing the same extraction procedure twice). The hot and room-temperature extracts were frozen at −80 °C and then lyophilized (Labconco Freezone 4.5®) at −50 °C to conserve the chemical characteristics of extracts. To reach the total volume of extract for all experiments, the leaves were collected in a period of 2 months (August to September), and the combined extracts were frozen with the freeze-drying process.

2.2 | Isolation of *Saprolegnia parasitica* strain

Fragments of the oomycete were removed from naturally infected *Colossoma macropomum* and then isolated, identified and stored at the Embrapa Tabuleiros Costeiros aquaculture laboratory. These pathogens were placed on a Petri dish containing 20 mL of potato dextrose agar (PDA) (Yao et al., 2017) and maintained in a germination chamber (Tecnal TE-40) at 25 °C for mycelial growth.

The identification of the isolated strain was confirmed using the PCR technique according to Hulvey et al. (2007) (GenBank KY418035).

2.3 | In vitro anti-oomycete activity on solid medium culture

A 9-mm disc from the oomycete culture was placed onto petri dishes (80 × 10 mm) containing PDA and different concentrations of extracts. This experiment used a factorial design (4 × 2 × 2) with four concentrations of extract (0.0, 2.5, 5.0 and 10 g/L), two extraction methods (80 °C and 27 °C extraction temperatures) and addition or not of chloramphenicol (500 mg/L). The antibiotic (chloramphenicol) was added in culture media of *Saprolegnia* to inhibit the bacterial growth. All treatments were performed in triplicate, and the experiment was observed for 96 h. The nominal concentrations of active compounds are presented in Table 1.

The mycelial growth was evaluated daily, measuring the perpendicular diameters using a digital pachymeter (150-mm LEE tools®). At the end of the experiment (96 h), all oomycete discs were removed and then placed onto new petri dishes without extract concentrations to confirm the fungicidal (no mycelial growth) or fungistatic effect (mycelial growth) (adapted from Corrêa et al., 2013).

The *Saprolegnia* sp. growth index (GI %) and the mycelial reduction (MR %) were calculated followed Kalatehjari et al. (2015):

$$\text{GI}\,(\%) = \frac{\text{Growth area of } \textit{Saprolegnia} \text{ sp. in the treatment group}}{\text{Growth area of } \textit{Saprolegnia} \text{ sp. in the control group}} \times 100$$

$$\text{MR}\,(\%) = 100 - \textit{Saprolegnia} \text{ sp. growth index}$$

TABLE 1 Active compounds in *Terminalia catappa* aqueous extracts based on HPLC (Meneses et al., 2020) used in in vitro antifungal tests against *Saprolegnia parasitica*

	Tested concentrations in vitro and weight of active compounds in each concentration					
	Hot extract			Room-temperature extract		
Active compounds	2.5 g/L	5.0 g/L	10.0 g/L	2.5 g/L	5.0 g/L	10.0 g/L
Gallic acid (mg)	18.40	36.80	73.60	8.125	16.25	32.50
Ellagic acid (mg)	23.85	47.70	95.40	7.05	14.10	28.20
α-Punicalagin (mg)	33.30	66.60	133.20	13.525	27.05	54.10
β-Punicalagin (mg)	57.17	114.35	228.70	18.40	36.80	73.60

2.4 | In vitro anti-oomycete activity on liquid medium culture

The hot extract was selected due to its superior results in the previous assay. Four 9-mm discs containing *Saprolegnia parasitica* were soaked in 50 mL of potato dextrose broth (PDB) and kept in a shaker (Cientec CT-712RN) at 125 rpm at 25 °C for 96 h.

This experiment was designed in a factorial scheme (4 × 4 × 2) with four hot extract concentrations (0.0, 2.5, 5.0 and 10 g/L), four exposure times (24, 48, 72 and 96 h), and the addition of chloramphenicol (0 and 500 mg/L); all treatments were performed in triplicate. At 24, 48, 72 and 96 h, one disc from each replicate was removed and then placed onto petri dishes with PDA without *T. catappa* extract to confirm the fungicidal (no mycelial growth) or fungistatic effect (mycelial growth). The *Saprolegnia* sp. growth index (GI%) and the mycelial reduction (MR%) were also calculated.

An acute toxicity assay on eggs was not conducted before because the acute fish toxicity of this plant extract was already achieved by Meneses et al. (2020).

2.5 | Preventive treatment of plant extract against pathogenic *S. parasitica* strain

This study obtained the approval of the Brazilian Agriculture Research Corporation ethical committee for animals (CEUA/00262019). In a previous in vivo assay, the effective concentrations in the in vitro assay showed toxicity against the eggs, so we reduced the concentrations, focusing on a prophylactic effect controlling the zoospores instead of mycelium.

For the in vivo assay, 15 couples of *P. scalare* provided the spawnings. Each couple was maintained in a 75-L aquarium plugged into a recirculating system (mechanic and biological filters). The couples were fed twice a day (Poytara® commercial diet, 32% crude protein); a substrate (ceramic tile) was available for reproduction.

Each spawning was transferred to a polyethylene tank (2 L) containing the same water as the parents' aquarium. The *Saprolegnia* infection occurred naturally and was monitored using photography every 24 h for 72 h.

TABLE 2 Active compounds in *Terminalia catappa* aqueous hot extract based on HPLC (Meneses et al., 2020) used in in vivo antifungal tests against *Saprolegnia parasitica*

	Tested concentrations in vivo and weight of active compounds in each concentration		
Active compounds	25 mg/L	50 mg/L	100 mg/L
Gallic acid (μg)	184	368	736
Ellagic acid (μg)	238	477	954
α-Punicalagin (μg)	333	666	1332
β-Punicalagin (μg)	571	1143	2287

Three concentrations of *T. catappa* extract (25, 50 and 100 mg/L) (Table 2) and two controls were distributed randomly in 30 spawnings. Each spawning represented a replicate, and six replicates per treatment were used. The water from the recirculation system was considered the negative control, and methylene blue at 1 mg/L was the positive control (Rahman et al., 2017).

The eggs were counted and classified as viable, unviable (dead and unfertilized), or infected (Oberlercher & Wanzenböck, 2016). The photographs were analysed with Image J® software. After 72 h, dead and alive larvae were also counted.

The physical–chemical water parameters of temperature (YSI 55-12FT®), dissolved oxygen (YSI 55-12FT®), pH (AKROM KR20®) and electrical conductivity (YSI 30-10FT®) were monitored daily. Total ammonia was monitored at the end of the experiment using a colorimetric kit.

2.6 | Statistical analysis

The data from in vitro tests were subjected to the Shapiro–Wilk normality test and then subjected to two-way ANOVA followed by the Tukey test ($p < 0.05$). Data from in vivo experiments were subjected to nonparametric Kruskal–Wallis rank test followed by the Dunn post hoc test. All statistical analysis was performed in SISVAR 5.6 and BioEstat 5.3 considering 5% ($p < 0.05$) for any significant difference (Zar, 2009).

3 | RESULTS

3.1 | In vitro anti-oomycete activity on solid medium culture

The hot extract achieved greater inhibition of the oomycete mycelial growth (Table 3; Figure 1). The highest concentration (10 g/L) was the most effective treatment for both extracts (room temperature 4.44 ± 3.09 mm diameter and hot temperature 0.00 ± 0.00 mm diameter) reaching 92.71% and 100% reduction respectively.

A synergism between the *T. catappa* aqueous extract and the antibiotic was observed when oomycete was exposed to 2.5 g/L extract; the medium culture with antibiotic presented twofold the mycelial reduction (75.66%) as that without antibiotic (36.83%) (Table 4; Figure 1). Nonetheless, increases in extract concentration (5 and 10 g/L), regardless of antibiotic addition, demonstrated anti-oomycete properties against *S. parasitica* with mycelial reduction >90%.

All treatments achieved effects on the mycelial growth compared with the control group at any exposure time. The antibiotic affected the growth of the *S. parasitica* in the control up to 48 h. A higher concentration of any extract inhibited *S. parasitica* growth at all times observed. However, the hot extract treatment at 10 g/L did not show any mycelial growth (Table 5; Figure 1).

The absence of mycelial growth observed at 10 g/L hot extract was confirmed as a fungicidal effect was present after growth in medium without extract. All other treatments had a fungistatic effect, presenting mycelial growth in medium culture without extract.

TABLE 3 Effect of *T. catappa* extracts on mycelial linear growth of the fish pathogenic *S. parasitica* oomycete strain after 96 h of exposure (solid medium)

Treatment of plant extract	Extracted at room temperature (27°C)			Extracted at hot temperature (80°C)		
Concentration (g/L)	Linear growth (mm)	*Saprolegnia* growth index (%)	Mycelial reduction (%)	Linear growth (mm)	*Saprolegnia* growth index (%)	Mycelial reduction (%)
0	60.98 ± 17.77 Ad	100.00	0.00	60.82 ± 17.68 Ad	100.00	0.00
2.5	27.46 ± 19.70 Bc	45.03	54.97	10.41 ± 5.67 Ac	17.12	82.88
5	6.97 ± 3.78 Ab	11.43	88.57	7.37 ± 3.58 Ab	12.12	87.88
10	4.44 ± 3.09 Ba	7.28	92.71	0.00 ± 0.00 Aa	0.00	100.00

Note: Different lowercase letters in the same column indicate significant differences between concentrations. Different uppercase letters in the same row indicate significant differences between extraction temperature ($p < 0.05$). *Saprolegnia* growth index (%): growth area of *Saprolegnia* sp. on the plate in the treatment group/growth area of *Saprolegnia* sp. on the plate in the control × 100; % Reduction: 100(%) − *Saprolegnia* growth index (%).

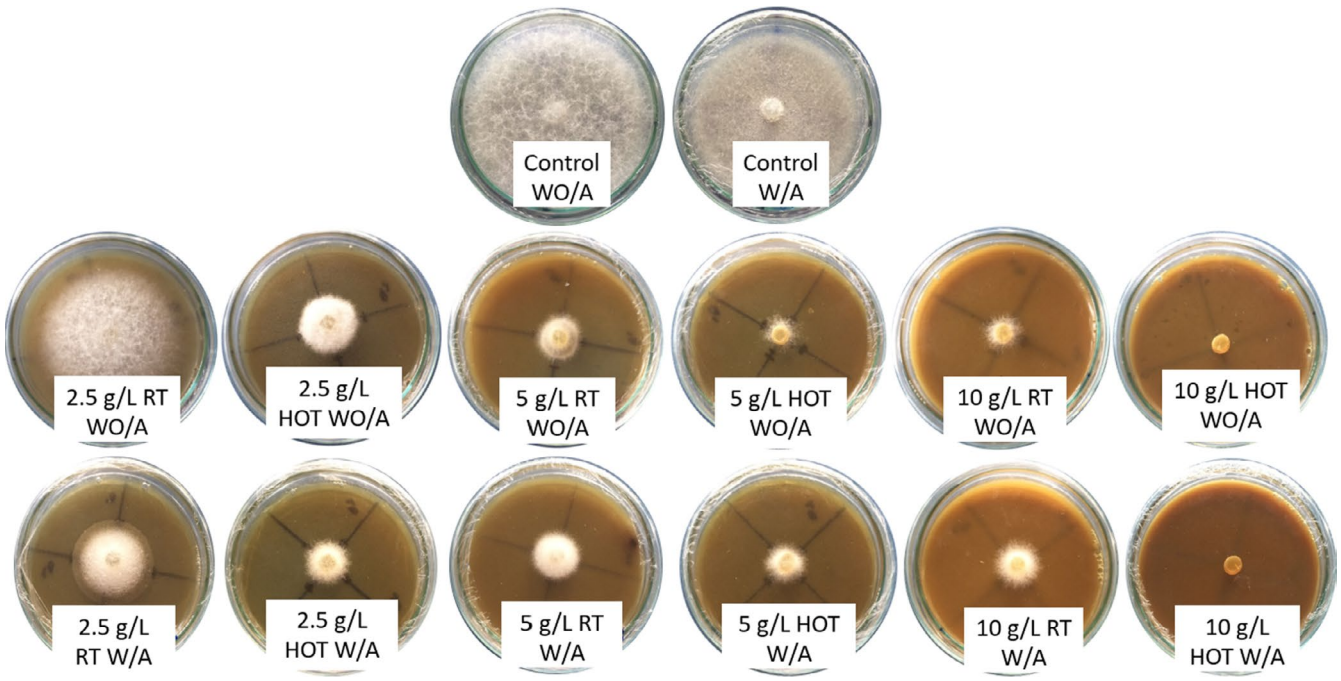

FIGURE 1 Anti-oomycete activity of different concentrations of *Terminalia catappa* extracts (Hot and Room Temperature) against *Saprolegnia parasitica* solid medium at 96 h in vitro. *WO/A: without antibiotic; W/A: with antibiotic (Chloramphenicol – 500 mg/L); RT: room temperature extract; HOT: hot extract

TABLE 4 Effect of *T. catappa* extracts with or without antibiotic on linear mycelial growth of the fish pathogenic *S. parasitica* oomycete strain after 96 h of exposure (solid medium)

Treatment of plant extract	WO/A medium			W/A medium		
Concentration (g/L)	Linear growth (mm)	*Saprolegnia* growth index (%)	Mycelial reduction (%)	Linear growth (mm)	*Saprolegnia* growth index (%)	Mycelial reduction (%)
0	65.73 ± 13.25 Bd	100.00	0.00	56.07 ± 20.10 Ad	85.30	14.70
2.5	24.21 ± 20.39 Bc	36.83	63.17	13.65 ± 9.89 Ac	24.34	75.66
5	7.18 ± 3.98 Ab	10.92	89.08	7.16 ± 3.37 Ab	12.77	87.23
10	1.58 ± 2.44 Aa	2.40	97.60	2.86 ± 3.61 Aa	5.11	94.89

Note: Different lowercase letters in the same column indicate significant differences between concentrations. Different uppercase letters in the same row indicate differences between type of culture medium ($p < 0.05$). WO/A—without antibiotic, W/A—with antibiotic (chloramphenicol – 500 mg/L). *Saprolegnia* growth index (%): growth area of *Saprolegnia* sp. on the plate in the treatment group/row area of *Saprolegnia* sp. on the plate in the control × 100; % Reduction: 100(%) − *Saprolegnia* growth index (%).

TABLE 5 Mycelial linear growth of *Saprolegnia parasitica* after 96 h of exposure to *Terminalia catappa* aqueous extract in solid medium culture

Conc. × Medium × Ext./Time		Mean ± *SD* (mm) Diameter 24 h	Mean ± *SD* (mm) Diameter 48 h	Mean ± *SD* (mm) Diameter 72 h	Mean ± *SD* (mm) Diameter 96 h
WO/A RT	0.0 g/L	43.37 ± 0.21 Af	73.29 ± 0.07 Bh	73.29 ± 0.07 Bf	73.29 ± 0.07 Bg
	2.5 g/L	11.42 ± 0.90 Ad	28.01 ± 2.03 Bf	46.08 ± 2.77 Ce	65.11 ± 3.10 Df
	5.0 g/L	1.98 ± 0.75 Aabc	4.29 ± 0.13 Abc	7.97 ± 0.51 Bb	12.91 ± 0.96 Cc
	10.0 g/L	0.89 ± 0.96 Aab	1.77 ± 1.18 Aab	3.14 ± 1.76 Aa	6.86 ± 1.46 Bb
WO/A HOT	0.0 g/L	43.16 ± 0.09 Af	73.16 ± 0.03 Bh	73.16 ± 0.03 Bf	73.16 ± 0.03 Bg
	2.5 g/L	3.61 ± 0.78 Abc	8.03 ± 0.19 Bd	13.18 ± 0.48 Cc	18.26 ± 1.74 Dd
	5.0 g/L	2.75 ± 0.27 Aabc	6.16 ± 1.05 Bcd	9.46 ± 0.94 Cb	11.98 ± 1.33 Dc
	10 g/L	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa
W/A RT	0.0 g/L	25.62 ± 1.09 Ae	51.84 ± 1.98 Bg	74.02 ± 1.35 Cf	73.18 ± 0.44 Cg
	2.5 g/L	4.26 ± 0.66 Ac	11.36 ± 1.99 Be	20.90 ± 4.93 Cd	32.53 ± 9.06 De
	5.0 g/L	2.73 ± 0.48 Aabc	6.33 ± 0.16 Bcd	8.15 ± 0.12 Bb	11.43 ± 0.69 Cc
	10.0 g/L	2.12 ± 0.35 Aabc	4.28 ± 0.21 ABbc	6.45 ± 0.28 Bb	10.05 ± 0.36 Cbc
W/A HOT	0.0 g/L	26.01 ± 0.57 Ae	50.9 ± 0.58 Bg	73.46 ± 0.03 Cf	73.46 ± 0.03 Cg
	2.5 g/L	2.68 ± 0.37 Aabc	7.37 ± 0.40 Bcd	13.02 ± 0.95 Cc	17.11 ± 1.53 Dd
	5.0 g/L	2.27 ± 0.18 Aabc	6.03 ± 0.34 Bcd	8.95 ± 0.81 Bc	11.40 ± 0.68 Dc
	10.0 g/L	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa

Note: Different lowercase letters in the same column indicate significant differences between treatments. Different uppercase letters in the same row indicates significant differences between exposure duration ($p < 0.05$). WO/A—without antibiotic, W/A—with antibiotic (Chloramphenicol – 500 mg/L), RT—room-temperature extract, HOT—hot extract and *SD*—standard deviation, Conc.—concentration, Ext.—extract.

3.2 | In vitro anti-oomycete activity on liquid medium culture

In liquid medium (PDB), concentrations of 5 and 10 g/L presented mycelial reduction in 100% at any observation time. The oomycete exposed to 5 and 10 g/L after reallocation to PDB medium showed no mycelial growth through 96 h, highlighting the fungicidal effect. The high degree of contact of the extract with the oomycete in liquid medium increased its effects (Tables 6 and 7).

3.3 | Preventive treatment against zoospore of *S. parasitica* on angelfish *P. scalare* eggs

The new extract concentrations used in the in vivo assay did not change any water parameters compared with the negative control (Table 8, $p < 0.05$). However, a slight decrease in dissolved oxygen and a slight increase in electrical conductivity were observed at the higher extract concentrations (50 and 100 mg/L).

A reduced number of viable eggs were observed in the negative control group; the opposite occurred at higher concentrations

TABLE 6 Effect of *T. catappa* extracts on linear mycelial growth of the fish pathogenic *S. parasitica* oomycete strain for different exposure times (liquid medium)

Conc./Exp.	24 h	48 h	72 h	96 h
0.0 g/L	67.96 ± 10.02 Ac	66.83 ± 11.77 Ac	65.87 ± 13.59 Ac	65.36 ± 15.07 Ab
2.5 g/L	45.54 ± 30.67 Cb	21.94 ± 29.15 Bb	18.76 ± 28.74 ABb	6.65 ± 19.91 Aa
5.0 g/L	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 000 Aa	0.00 ± 0.00 Aa
10.0 g/L	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa

Note: Different lowercase letters in the same column indicate significant differences between concentrations. Different uppercase letters in the same row indicate significant differences between exposure duration ($p < 0.05$). Conc.—concentration, Exp.—exposure.

TABLE 7 Mycelial reduction of the fish pathogenic *S. parasitica* strain for different exposure times to *T. catappa* hot extract in liquid medium

	24 h		48 h		72 h		96 h	
Conc./Exp.	*Saprolegnia* growth index (%)	Mycelial reduction (%)	*Saprolegnia* growth index (%)	Mycelial reduction (%)	*Saprolegnia* growth index (%)	Mycelial reduction (%)	*Saprolegnia* growth index (%)	Mycelial reduction (%)
0.0 g/L	100	0	100	0	100	0	100	0
2.5 g/L	67.01	32.99	32.83	67.17	28.48	71.52	10.17	89.83
5.0 g/L	0	100	0	100	0	100	0	100
10.0 g/L	0	100	0	100	0	100	0	100

Note: Saprolegnia growth index (%): growth area of *Saprolegnia* sp. on the plate in the treatment group/growth area of *Saprolegnia* sp. on the plate in the control × 100; % Reduction: 100(%) − *Saprolegnia* growth index (%).

TABLE 8 Certain quality parameters of water in incubators containing *Pterophyllum scalare* eggs after exposure to *Terminalia catappa* aqueous extract

Treatments (mg/L)	Temperature (°C)	pH	Electrical conductivity (mS/cm)	Dissolved oxygen (mg/L)
Negative control	26.03 ± 1.26 a	7.03 ± 0.60 a	0.16 ± 0.03 ab	7.21 ± 0.97 ab
Positive control	25.93 ± 1.16 a	7.13 ± 0.35 a	0.17 ± 0.01 b	6.30 ± 0.56 b
25	25.37 ± 0.79 a	7.41 ± 0.34 a	0.15 ± 0.01 a	7.73 ± 0.54 a
50	25.76 ± 0.86 a	7.14 ± 0.07 a	0.16 ± 0.01 b	6.20 ± 1.29 b
100	25.86 ± 0.62 a	6.89 ± 0.41 a	0.17 ± 0.01 b	6.24 ± 0.68 b

Note: Different letters in the same column indicate significant differences between treatments ($p < 0.05$).

(50 and 100 mg/L) at 24 h and 48 h (Figure 2a,b). After 72 h, there were no statistical differences between treatments ($p > 0.05$). Nonetheless, 72 h corresponds to the natural hatching time of *P. scalare* eggs (Figure 2c).

The number of viable eggs showed an inverse correlation with that of infected eggs. After 72 h, all eggs died in the negative control; the contrary was observed in other treatments. After 24 h (Figure 3a), no differences were observed between treatments. However, the number of infected eggs increased over time (Figure 3b,c). On the other hand, exposure to 50 and 100 mg/L exhibited a preventive effect, with less than 10% of eggs infected after 72 h (Figure 3c). The positive control obtained similar results to those of extract concentrations. However, the standard deviation was higher in this treatment, demonstrating a heterogeneous response to *Saprolegnia* infection.

The lower percentage of infected eggs promoted a higher number of larvae at higher extract concentrations (50 and 100 mg/L), demonstrating a great degree of hatchability (Figure 4a). The extract concentration of 25 mg/L and the positive control presented a heterogeneous effect related to viable larvae (Figure 4b) and dead larvae (Figure 4c), demonstrated using the high standard deviation.

4 | DISCUSSION

The oomycete *S. parasitica* is responsible for high mortality and economic losses in the ornamental fish and food fish industry (Shaalan et al., 2018). Among these infections, saprolegniosis is one of the most aggressive and problematic diseases for several wild and farmed species of fish in different life stages, especially eggs (Songe et al., 2016). The pathogen hyphae break the chorionic membrane that regulates the osmosis process of the embryo (Liu et al., 2014), causing its mortality, and consequent production losses.

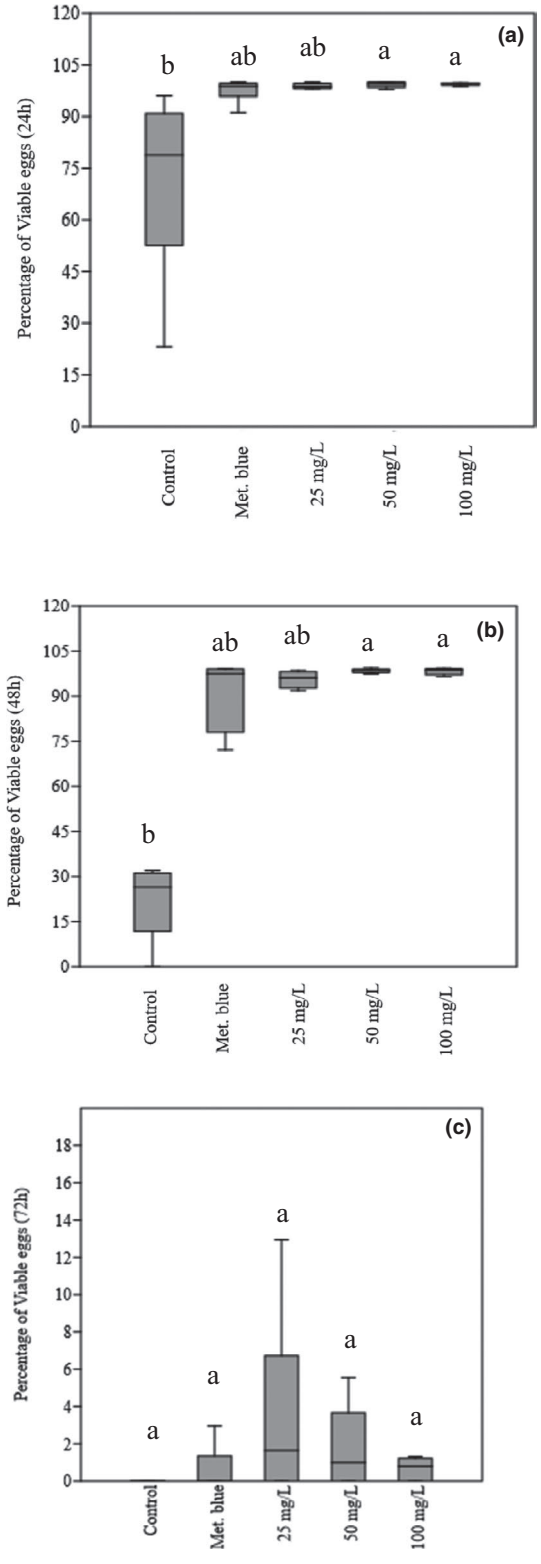

FIGURE 2 Percentage of viable *Pterophyllum scalare* eggs after exposure to *Terminalia catappa* extract. (a) 24 h, (b) 48 h, (c) 72 h

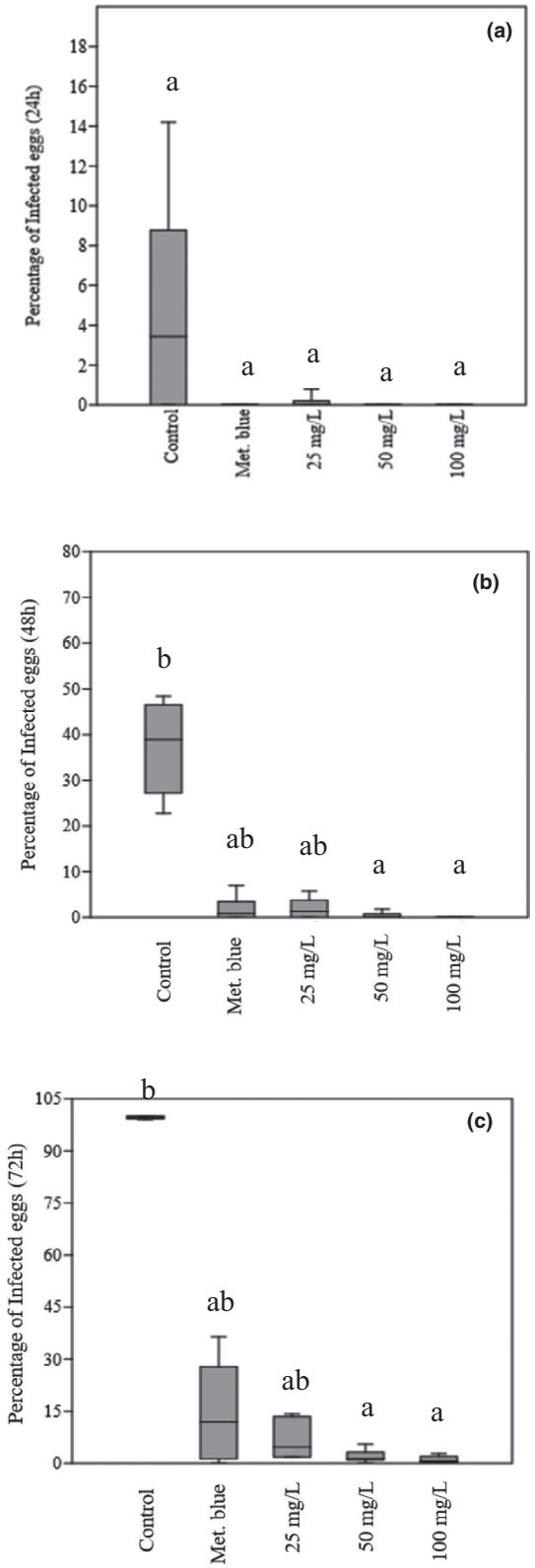

FIGURE 3 Percentage of *Pterophyllum scalare* eggs infected by *Saprolegnia parasitica* after exposure to *Terminalia catappa* extract. (a) 24 h, (b) 48 h, (c) 72 h

The present study evidenced synergism between *T. catappa* and chloramphenicol in in vitro assay. This effect of chloramphenicol has already been reported in oomycetes (Rooke, & Shattock, 1983) and may have helped the plant extract to control the oomycete. Synergic effect of plant extract and other drugs is poorly reported in the literature. The synergic effect of *T. catappa* extract has reported with antifungals as nystatin and amphotericin B (Chanda et al., 2013; Mendonça et al., 2020).

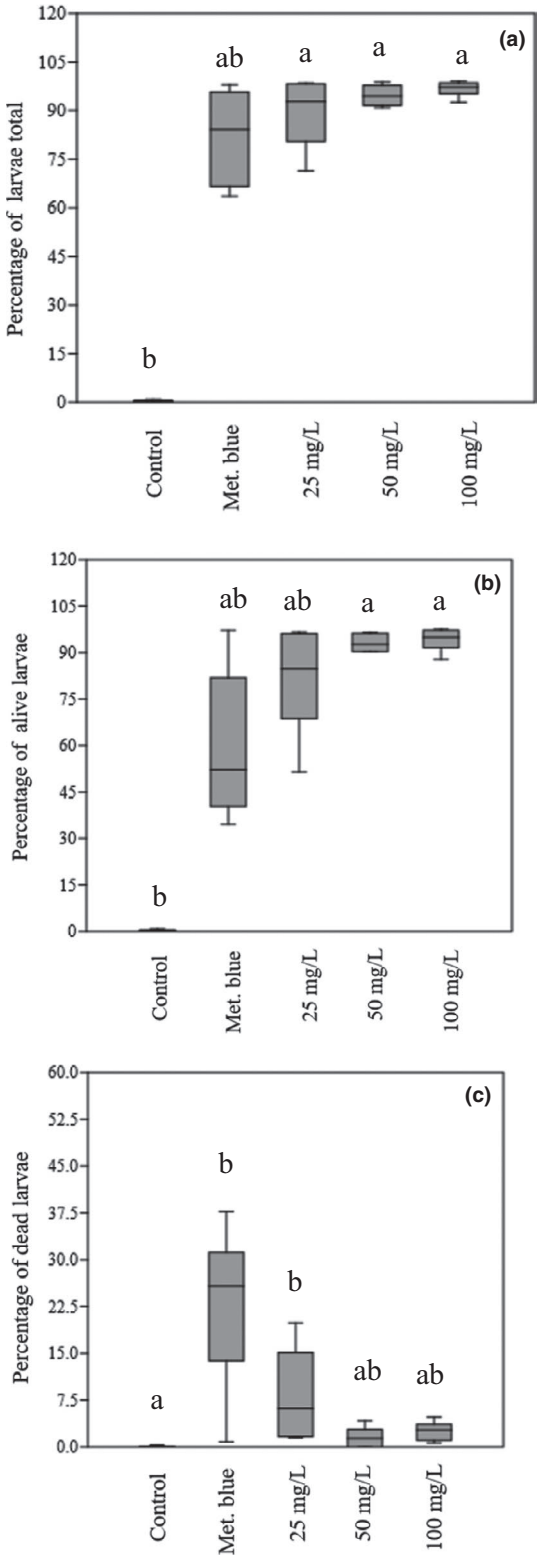

FIGURE 4 Percentage of *Pterophyllum scalare* larvae after exposure to *Terminalia catappa* extract. (a) Total larvae, (b) Alive larvae, (c) Dead larvae

Studies with *T. catappa* extract usually used extraction at room temperature (Claudiano et al., 2009, 2012), but hot extraction promoted higher content of active compounds (Jeyaseelan, & Jashothan, 2012; Meneses et al., 2020); then, the difference observed in the present study in vitro reflected the greater efficacy of the hot extract on the mycelium of *S. parasitica*.

The angelfish egg mortality caused by oomycete infections is a problem to captivity rearing and many chemicals are used inappropriately to control it, affecting the environment, fish and human. The herbal medicines have been increasingly studied to replace the chemotherapeutics due their the positive aspects as biodegradation, less toxicity and availability (Eissa et al., 2013; Saha et al., 2017; Ventura et al., 2019).

The extract of *T. catappa* presented potential for use in aquaculture, promoting stress reduction (Maftuch et al., 2018), increasing the survival and growth of fish larvae (Nurhidayat et al., 2016), improving reproductive activity (Lee et al., 2016), improving the haematological profile (Nugroho et al., 2017), increasing the hatching rate for fish eggs and providing a natural food such as *Paramecium* (Sung, & Abol-Munafi, 2019). However, the concentrations tested in vitro against *S. parasitica* mycelium were toxic to the eggs, so new concentrations were evaluated in in vivo tests. These were effective in controlling oomycete in *P. scalare* eggs, preventing zoospore infection.

The difference of efficacy between concentrations against life stages (mycelium or zoospore) of *S. parasitica* has been reported. Zoospores are more sensitive than mycelium, and lower concentrations would be sufficient to prevent saprolegniosis (Sun et al., 2014; Xue-Gang et al., 2013).

During the in vivo experiment, the reduction in dissolved oxygen was due to the degradation of organic matter in the extract (Claudiano et al., 2012) and the presence of tannins, which also increase electrical conductivity (Miksen, & Media, 2016). However, these values remained within the acceptable level for angelfish farming (Çağiltay et al., 2017).

The efficacy of *T. catappa* extract to prevent infection in eggs is related to the presence of active compounds including tannins, which inhibit enzymes and proteins in the cell membrane (Cowan, 1999), and phenolic compounds, such as gallic and ellagic acids, and α- and β-punicalagin, which damage the membrane and affect the metabolic activity of cells (Hili et al., 1997).

The efficacy of hot extract is due to the extraction of more compound (flavonoids and tannins) than at room temperature. The greater egg hatchability is associated with the tannin content of the extract, which protects the eggs and even prevents infection by *Saprolegnia* sp., improving the hatching process (Saenal et al., 2020). Hot extracts of *T. catappa* produced at 50 °C improved the egg hatching rate of *Chana striata* at 30 mg/L (hatching ratio 50 ± 3.33%) (Basir, & Kaharuddin, 2020). Other plant extracts such as *Bixa Orellana*, *Piper betle* and *Phyllanthus niruri* produced in temperatures of 80–90 °C/2 h at 50 mg/L increase the hatching rate of *Danio rerio* eggs (Lamban et al., 2019; Vera et al., 2016).

Each fish species has different tolerance to extracts, and different concentrations should be tested because high concentrations can increase mortality and cause morphologic alterations. In this work, no abnormalities in the larvae were observed, demonstrating

that hot *T. catappa* extract does not affect the morphology and integrity of the hatched larvae. These results corroborate results found for *Betta splendens* where no deformities were reported when larvae were exposed to a *T. catappa* concentration of 0.5 g/L (Waris et al., 2018).

Thus, plant extracts are an alternative to control this oomycete in fish eggs, mainly as a prophylactic measure. The extraction process is an important step to adjust the extraction of the compounds, and the applied concentration should be determined based on the fish species and the life stage of the pathogen.

5 | CONCLUSION

T. catappa aqueous hot extract at the concentrations of 50 and 100 mg/L was most effective to prevent the growth of *S. parasitica* zoospores. The concentration of 50 mg/L is an adequate prophylactic measure to prevent *Saprolegnia* infection on *P. scalare* eggs without affecting the viability and hatching rate, as well as the survival and morphology of the larvae.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

All authors listed executed substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; and drafting the manuscript or critically revising it for important intellectual content; and final approval of the version to be published; and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon reasonable request by the corresponding author.

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REFERENCES

Abiodun, O. O., Rodríguez-Nogales, A., Algieri, F., Gomez-Caravaca, A. M., Segura-Carretero, A., Utrilla, M. P., Rodriguez-Cabezas, M. E., & Galvez, J. (2016). Antiinflammatory and immunomodulatory activity of an ethanolic extract from the stem bark of *Terminalia catappa* L. (Combretaceae): *In vitro* and *in vivo* evidences. *Journal of Ethnopharmacology*, *192*, 309–319. https://doi.org/10.1016/j.jep.2016.07.056.

Amiri, H., & Meshkini, S. (2019). Antifungal effects of *Achillea officinolis* and *Matricaria chamomilla* plant extracts on control infection of rainbow trout eggs by *Saprolegnia parasitica*. *Veterinary Research*, *15*(1), 97–107. https://doi.org/10.21270/archi.v8i12.4654.

Basir, B., & Kaharuddin, K. (2020). Effectiveness of Ketapang leaf extract (*Terminal capta* L) in water media on crude egg hatching. *International Journal on Advanced Science, Education, and Religion*, *3*(1), 33–39. https://doi.org/10.33648/ijoaser.v3i1.44.

Çağiltay, F., Atanasoff, A., & Nikolov, G. (2017). Influence of stocking density on some water quality parameters and growth traits in angel fish (*Pterophyllum skalare*). *Lucrări Științifice-Universitatea De Științe Agricole Și Medicină Veterinară, Seria Zootehnie*, *69*, 144–148.

Chambel, J., Costa, R., Gomes, M., Mendes, S., Baptista, T., & Pedrosa, R. (2014). Hydrogen peroxide, iodine solution and methylene solution highly enhance the hatching rate of freshwater ornamental fish species. *Aquaculture International*, *22*(6), 1743–1751. https://doi.org/10.1007/s10499-014-9779-1.

Chanda, S., Rakholiya, K., Dholakia, K., & Baravalia, Y. (2013). Antimicrobial, antioxidant, and synergistic properties of two nutraceutical plants: *Terminalia catappa* L. and *Colocasia esculenta* L. *Turkish Journal of Biology*, *37*(1), 81–91. https://doi.org/10.3906/biy-1203-41.

Chitmanat, C., Tongdonmuan, K., & Nunsong, W. (2005). The use of crude extracts from traditional medicinal plants to eliminate *Trichodina* sp. in tilapia (*Oreochromis niloticus*) fingerlings. *Songklanakarin Journal of Science and Technology*, *27*, 359–364.

Claudiano, G. D. S., Neto, J. D., Sakabe, R., Cruz, C. D., Salvador, R., & Pilarski, F. (2009). Eficácia do extrato aquoso de *Terminalia catappa* em juvenis de tambaqui parasitados por monogenéticos e protozoários. *Revista Brasileira De Saúde E Produção Animal*, *10*(3), 625–636.

Claudiano, G. S., Pilarski, F., Cruz, C., Salvador, R., de Andrade Belo, M. A., & Moraes, F. R. (2012). Concentraçao letal CL50 do extrato aquoso de folhas de *Terminalia catappa* em guaru, *Phalloceros caudimaculatus*. *Archives of Veterinary Science*, *17*(3), 15–19. https://doi.org/10.5380/avs.v17i3.23168.

Corrêa, B. F., Stohl, F. E., Robaldo, R. B., & Pereira, D. I. B. (2013). In vitro effect of chemical on hyphal growth of *Saprolegnia* spp. *Ciência Rural*, *43*(6), 1021–1024. https://doi.org/10.1590/S0103-84782013005000074.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology. Reviews*, *12*(4), 56–64. https://doi.org/10.1128/CMR.12.4.564.

de Vera, J. S., De Castro, M. E. G., & Dulay, R. M. R. (2016). Phytochemical constituents and teratogenic effect of lyophilized extracts of *Bixa orellana* L. (Achuete) and *Piper betle* L. (Ikmo) leaves in *Danio rerio* embryos. *Der Pharma Chemica*, *8*(18), 432–437.

Eissa, A. E., Abdelsalam, M., Tharwat, N., & Zaki, M. (2013). Detection of *Saprolegnia parasitica* in eggs of angelfish *Pterophyllum scalare* (Cuvier–Valenciennes) with a history of decreased hatchability. *International Journal of Veterinary Science and Medicine*, *1*(1), 7–14. https://doi.org/10.1016/j.ijvsm.2013.04.001.

Fuangsawat, W., Abking, N., & Lawhavinit, O. A. (2011). Sensitivity comparison of pathogenic aquatic fungal hyphae to sodium chloride, hydrogen peroxide, acetic acid and povidone iodine. *Kasetsart Journal-Natural Science*, *45*, 84–89.

Hayaza, S., Istiqomah, S., Susilo, R. J. K., Inayatillah, B., Ansori, A. N. M., Winarni, D., & Darmanto, W. (2019). Antidiabetic activity of ketapang (*Terminalia catappa* L.) leaves extract in streptozotocin-induced diabetic mice. *Indian Veterinary Journal*, *96*(12), 11–13.

Hili, P., Evans, C. S., & Veness, R. G. (1997). Antimicrobial action of essential oils: The effect of dimethylsulphoxide on the activity of cinnamon oil. *Applied Environmental Microbiology*, *24*, 269–275. https://doi.org/10.1046/j.1472-765x.1997.00073.x.

Hulvey, J. P., Padgett, D. E., & Bailey, J. C. (2007). Species boundaries within *Saprolegnia* (Saprolegniales, Oomycota) based on morphological and DNA sequence data. *Mycologia*, *99*(3), 421–429. https://doi.org/10.1080/15572536.2007.11832567.

Humsari, A., Rosidah, & Junianto (2017). Effectiveness of *Kaempferia galanga* extract for the prevention of saprolegniasis on catfish *Clarias gariepinus* eggs. *Jurnal Akuakultur Indonesia*, *16*(1), 1–7. https://doi.org/10.19027/jai.16.1.1-7.

Jeyaseelan, E. C., & Jashothan, P. J. (2012). In vitro control of *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (ATCC 25922) by *Ricinus communis* L. *Asian Pacific Journal of Tropical Biomedicine*, *2*(9), 717–721. https://doi.org/10.1016/S2221-1691(12)60216-0.

Kalatehjari, P., Yousefian, M., & Khalilzadeh, M. A. (2015). Assessment of antifungal effects of copper nanoparticles on the growth of the fungus *Saprolegnia* sp. on white fish (*Rutilus frisii kutum*) eggs. *The Egyptian Journal of Aquatic Research*, *41*(4), 303–306. https://doi.org/10.1016/j.ejar.2015.07.004.

Khemis, I. B., Aridh, N. B., Hamza, N., M'Hetli, M., & Sadok, S. (2016). Antifungal efficacy of the cactaceae *Opuntia stricta* (Haworth) prickly pear ethanolic extract in controlling pikeperch *Sander lucioperca* (Linnaeus) egg saprolegniasis. *Journal of Fish Diseases*, *39*, 377–383. https://doi.org/10.1111/jfd.12356.

Lamban, I., Balbeuna, E., Lee, M., Sacdalan, M., arpio JR., A., & Cabuhat, K. S. (2019). Toxic and teratogenic effects of Sampa-Sampalukan (*Phyllanthus niruri*) leaves extract using *Danio rerio* embryo assay. *Egyptian Academic Journal of Biological Sciences*, *B. Zoology*, *11*(1), 109–115. https://doi.org/10.21608/eajbsz.2019.30839.

Lee, S. W., Farhan, R., Wendy Wee, W. Z. M., & Ibrahim, C. O. (2016). The effects of tropical almond *Terminalia catappa* L., leaf extract on breeding activity of Siamese Gourami, *Trichogaster pectoralis*. *International Journal of Fisheries and Aquatic Studies*, *4*(4), 431–433.

Liu, Y., De Bruijn, I., Jack, A. L., Drynan, K., Van Berg Den, A. H., Thoen, E., Sandoval-Sierra, V., Skaar, I., Van West, P., Dieguez-Uribeondo, J., Van Voort Der, M., Mendes, R., Mazzola, M., & Raaijmakers, J. M. (2014). Deciphering microbial landscapes of fish eggs to mitigate emerging diseases. *ISME Journal*, *8*(10), 2002–2014. https://doi.org/10.1038/ismej.2014.44.

Lusiastuti, M., Taukhid, T., Anggi, I., & Caruso, D. (2017). Dry green leaves of Indian almond (*Terminalia catappa*) to prevent streptococcal infection in juveniles of the Nile tilapia (*Oreochromis niloticus*). *Bulletin of the European Association of Fish Pathologists*, *37*(3), 119–125.

Maftuch, M., Suprastyani, H., Sanoesi, E., Putra, G. A., Putra, M. F., & Prihanto, A. A. (2018). The effects of Ketapang (*Terminalia catappa*) Bark crude extract on inhibition of *Aeromonas hydrophila* growth and blood cells of the infected carp (*Cyprinus carpio*). *Rekayasa*, *11*(2), 87–94. https://doi.org/10.21107/rys.v11i2.4414.

Mendonça, A. M. S., Rocha, C. H. L., Campos, C. D. L., Barros Filho, A. K. D., da Silva, L. C. N., dos Santos, J. R. A., Rocha, F. M. G., & de Andrade Monteiro, C. (2020). Synergism between fluconazole and *Terminalia catappa* extract and its use in the treatment of *Candida*-infected *Tenebrio molitor* larvae. *Brazilian Journal of Development*, *6*(10), 78929–78950. https://doi.org/10.34117/bjdv6n10-358.

Meneses, J. O., dos Santos Cunha, F., Dias, J. A. R., da Cunha, A. F. S., dos Santos, F. J., da Costa Sousa, N., do Couto, M. V. S., Paixão, P. E. G., Abe, H. A., dos Santos Lima, B., de Carvalho Neto, A. G., de Souza Araújo, A. A., da Costa, L. P., Cardoso, J. C., & Fujimoto, R. Y. (2020). Acute toxicity of hot aqueous extract from leaves of the *Terminalia catappa* in juvenile fish *Colossoma macropomum*. *Aquaculture International*, *28*, 2379–2396. https://doi.org/10.1007/s10499-020-00596-z.

Miksen, C., & Media, D. (2016, June 2020). *Aquariums: How does tannic acid affect aquariums?*. http://pets.thenest.com/tannic-acid-affect-aquariums-12501.html

Mininel, F. J., Leonardo Junior, C. S., Espanha, L. G., Resende, F. A., Varanda, E. A., Leite, C. Q. F., Vilegas, W., & dos Santos, L. C. (2014). Characterization and quantification of compounds in the hydroalcoholic extract of the leaves from *Terminalia catappa* Linn. (Combretaceae) and their mutagenic activity. *Journal Evidence-Based Complementary Alternative Medicine*, *2014*, 1–11. https://doi.org/10.1155/2014/676902.

Nugroho, R. A., Manurung, H., Nur, F. M., & Prahastika, W. (2017). *Terminalia catappa* L. extract improves survival, hematological profile and resistance to *Aeromonas hydrophila* in *Betta* sp. *Archive of Polish Fisheries*, *25*(2), 103–115. https://doi.org/10.1515/aopf-2017-0010.

Nurhidayat, N., Wardin, L., & Sitorus, E. (2016). The survival and growth performance of juvenile cardinal tetra (*Paracheirodon axelrodi*) with application of tropical almond (*Terminalia catappa*) leaves. *Nusantara Bioscience*, *8*(1), 1–4. https://doi.org/10.13057/nusbiosci/n080101.

Oberlercher, T. M., & Wanzenböck, J. (2016). Impact of electric fishing on egg survival of whitefish, *Coregonus lavaretus*. *Fisheries Management and Ecology*, *23*(6), 540–547. https://doi.org/10.1111/fme.12197.

Özçelik, H., Taştan, Y., Terzi, E., & Sönmez, A. Y. (2020). Use of onion (*Allium cepa*) and garlic (*Allium sativum*) wastes for the prevention of fungal disease (*Saprolegnia parasitica*) on eggs of rainbow trout (*Oncorhynchus mykiss*). *Journal of Fish Diseases*, *43*(10), 1325–1330. https://doi.org/10.1111/jfd.13229.

Phillips, A. J., Anderson, V. L., Robertson, E. J., Secombes, C. J., & Van West, P. (2008). New insights into animal pathogenic oomycetes. *Trends in Microbiology*, *16*(1), 13–19. https://doi.org/10.1016/j.tim.2007.10.013.

Pinheiro Silva, L., Damacena de Angelis, C., Bonamin, F., Kushima, H., José Mininel, F., Campaner dos Santos, L., Karina Delella, F., Luis Felisbino, S., Vilegas, W., Regina Machado da Rocha, L., Aparecido dos Santos Ramos, M., Maria Bauab, T., Toma, W., & Akiko Hiruma-Lima, C. (2015). *Terminalia catappa* L.: A medicinal plant from the Caribbean pharmacopeia with anti-*Helicobacter pylori* and antiulcer action in experimental rodent models. *Journal of Ethnopharmacology*, *159*, 285–295. https://doi.org/10.1016/j.jep.2014.11.025.

Rahman, M. A., Rahman, M. H., Yeasmin, S. M., Asif, A. A., & Mridha, D. (2017). Identification of causative agent for fungal infection and effect of disinfectants on hatching and survival rate of Bata (Labeo Bata) larvae. *Advances in Plants & Agriculture Research*, *7*(4), 342–349. https://doi.org/10.15406/apar.2017.07.00264.

Rooke, D. M., & Shattock, R. C. (1983). Effect of chloramphenicol and streptomycin on developmental stages of *Phytophthom infestans*. *Microbiology*, *129*(11), 3401–3410. https://doi.org/10.1099/00221287-129-11-3401.

Saenal, S., Yanto, S., & Amirah, A. (2020). Perendaman Telur dalam Larutan Daun Ketapang (*Terminalia cattapa* L) Terhadap Daya Tetas Telur

Ikan Mas (*Cyprinus carpio* L). *Jurnal Pendidikan Teknologi Pertanian*, *6*(1), 115–124. https://doi.org/10.26858/jptp.v6i1.11376.

Saha, S., Behera, S., Bhakta, D., Das, A., Mandal, A., Kumar, S., & Mondal, A. (2017). Piscicidal effect of *Luffa cylindrica* fruit extract on tilapia fingerlings, *Oreochromis mossambicus* in captive condition. *Journal of Pharmacognosy and Phytochemistry*, *6*(3), 427–430.

Shaalan, M., El-Mahdy, M., Saleh, M., & El-Matbouli, M. (2018). Aquaculture in Egypt: Insights on the current trends and future perspectives for sustainable development. *Reviews in Fisheries Science & Aquaculture*, *26*(1), 99–110. https://doi.org/10.1080/23308249.2017.1358696.

Songe, M., Willems, A., Wiik-Nielsen, J., Thoen, E., Evensen, Ø., Van West, P., & Skaar, I. (2016). *Saprolegnia diclina* IIIA and *S. parasitica* employ different infection strategies when colonizing eggs of Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases*, *39*(3), 343–352. https://doi.org/10.1111/jfd.12368.

Sun, Q., Hu, K., & Yang, X. L. (2014). The efficacy of copper sulfate in controlling infection of *Saprolegnia parasitica*. *Journal of World Aquaculture Society*, *45*(2), 220–225. https://doi.org/10.1111/jwas.12113.

Sung, Y. Y., & Abol-Munafi, A. B. (2019). *Terminalia catappa* leaf extract is an effective rearing medium for larviculture of gouramis. *Journal of Applied Aquaculture*, *32*(2), 175–185. https://doi.org/10.1080/10454438.2019.1614509.

Venkatalakshmi, P., Brindha, P., & Vellingiri, V. (2015). In vitro antioxidant and anti-inflammatory studies on bark, wood and fruits of *Terminalia catappa* L. *International Journal of Phytomedicine*, *7*(3), 246–253.

Ventura, A. S., de Castro Silva, T. S., Cardoso, C. A. L., & Inoue, L. A. K. A. (2019). Características do anestésico alternativo de erva cidreira (*Lippia alba*) e alecrim pimenta (*Lippia sidoides*) em peixes. *Medicina Veterinária*, *13*(3), 416–428. https://doi.org/10.26605/medvet-v14n3-3304.

Waris, A., Mansyur, K., & Rusaini, R. (2018). Penggunaan Bubuk Daun Ketapang (*Terminalia catappa*) dengan Dosis dan Suhu Inkubasi Berbeda Terhadap Embriogenesis dan Penetasan Telur Ikan Cupang (*Betta splendens*). *Proceeding Simposium Nasional Kelautan Dan Perikanan*, *5*, 9–24.

Xue-Gang, H., Lei, L., Cheng, C., Kun, H., Xian-Le, Y., & Gao-Xue, W. (2013). In vitro screening of Chinese medicinal plants for antifungal activity against *Saprolegnia* sp. and *Achlya klebsiana*. *North American Journal of Aquaculture*, *75*(4), 468–473. https://doi.org/10.1080/15222055.2013.808298.

Yao, J. Y., Lin, L. Y., Yuan, X. M., Ying, W. L., Xu, Y., Pan, X. Y., & Ge, P. H. (2017). Antifungal activity of Rhein and aloe-emodin from *Rheum palmatum* on fish pathogenic *Saprolegnia* sp. *Journal of the World Aquaculture Society*, *48*(1), 137–144. https://doi.org/10.1111/jwas.12325.

Zar, J. H. (2009). *Biostatistical analysis*. Prentice-Hall.

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