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# Is there antimicrobial property of coconut oil and lauric acid against fish pathogen?

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ABSTRACT

This study evaluated in vitro the antimicrobial activity of virgin coconut oil (VCO) and lauric acid (LA) against three fish pathogens (*Aeromonas hydrophila, Saprolegnia parasitica* and *Ichthyophthirius multifiliis*). The experiments occurred in completely randomized design with five concentration for pathogen to determine lethal concentration. All data were subjected to analysis of variance (ANOVA) with post-hoc Tukey test ($p \leq 0.05$). Virgin coconut oil (VCO) and lauric acid (LA) affected the fungal and bacterial growth. Only the lauric acid (LA) prevent the mycelial growth ($r^2 = 0.94$) while the virgin coconut oil (VCO) reduced it. However, none treatment (VCO and LA) promoted fungicidal effect. Lauric acid provoked complete mortality (100%) of *Ichthyophthirius multifiliis* at concentration 40 $mg.L^{-1}$ while the virgin coconut oil only reduced its development with 386.71 $\mu L.L^{-1}$ (equivalent to the LA 200 $mg.L^{-1}$). In the bacterial assy, both VCO and LA caused reduced the colonies amount, but they have no any inhibition halo against the bacterium. The results suggest positive effect to control the pathogen development with greater effects using lauric acid.

## 1. Introduction

Intensive fish production has engendered problems with diseases in aquatic organisms owing to ruptures in the parasite-host environment relationship, elevating infection and the spread of fish pathogens (Moraes and Martins, 2004; Val et al., 2004).

Some fish pathogens can rapidly cause mortality in fish and economic losses for fish farmers due to the need for costly treatments (Soltani et al., 1996). Various chemical products and antibiotics are used by fish farmers, sometimes without adequate knowledge, provoking environmental problems owing to resistant microorganisms (Suhet et al., 2011).

Consequently, in order to ensure the security of the environment and human health, antibiotics are now had reduced use in animal production (Mello et al., 2013). In particular, eco-friendly alternatives have been necessary to avoid chemicals for treatment (Rayan et al., 2005; Nakatsuji et al., 2009; Yang and Wu, 2013).

Some recent studies reported the antimicrobial activity of virgin coconut oil (VCO) for human and animal health. Its positive effects owe to the fact it contains medium-chain fatty acids, especially lauric acid.

Reports about coconut oil and lauric acid (LA) used against different human pathogens does exit, such as fungicide effect for *Candida* sp., antiprotozoan in *Giardia duodenalis* (Rayan et al., 2005), fungistatic for *Aspergillus* and *Penicillium* sp. (Altieri et al., 2007) as well as bactericidal and bacteriostatic for *Staphylococcus aureus*, *Clostridium* sp., *Escherichia coli* and *Salmonella* sp. (Rouse et al., 2005; (Shilling et al., 2013; Skřivanová et al., 2006; Timbermont et al., 2011).

However, scientific papers on the use of coconut oil and LA to control fish pathogens remain missing. For this reason and searching for a different alternative to treatments with chemicals, this study evaluated

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in vitro the antimicrobial activity of VCO and LA against three fish pathogens.

## 2. Material and method

### 2.1. Virgin coconut (VCO) and lauric acid (LA)

Virgin coconut oil (VCO) undergone fat acid extraction (ester type) following the methodology from (Schutter and Dick, 2000).

Chemical analysis of VCO was carried out via gas chromatography (GCMS-QP5000, Shimadzu), using a spectrometer of mass at electronic ionization. The VCO reacted with Hexane P.A. 2 mL plus methanolic solution KOH 2 M 200 μL and was stirred for 30 s. Afterwards, the sample received saturated saline solution (NaCl 3 mL), generating the superior phase of metallic esters and fat acids. The solution with metallic esters was diluted 1000 times and then placed into vials for gas chromatographic analysis.

Specific conditions were set as the temperature of the injector (200 °C), the temperature of the detector (250 °C), the gas transporter, helium, caudal 1 mL/min in a split ratio of 1:40 according to Wang et al. (2020) (adapted methodology).

The oven begins at 50 °C (isothermal for 10 min), heating two degrees per minute until 200 °C and then 10 degrees per minute until 290 °C (isothermal for 10 min). The parameters of the columns were id 0.25 mm, length 30 mm and thickness 0.25 mm. The spectrometric conditions were ionized tension 70 eV, sweep rate of one sweep/s, and gamma of mass *m/z* between 50 and 400.

The compounds were identified through the injection of normalization comparing the retention times. Lauric acid (LA) standard ($C_{12}H_{24}O_2$; Sigma-Aldrich) diluted into HEXANE PA 100 ppm was used for the quantitative analysis of LA at concentrations of 6.25, 12.50, 25.00, 50.00 and 100.00 mg.$L^{-1}$. The data were analyzed with the aid of XCALIBUR software.

### 2.2. Obtaining pathogenic strains

After chromatographic analysis, the study evaluated the effects in vitro of VCO and LA against three fish pathogens: *Aeromonas hydrophila* (CPQBA22808) previously isolated from Surubim (*Pseudoplatystoma* sp.) (Silva et al., 2012) and trophonts of *Ichthyophthirius multifiliis* and *Saprolegnia parasitica* from naturally infested tambaqui *Colossoma macropomum* (Fu et al., 2014; adapted from Corrêa et al., 2013).

## 3. In vitro assays

### 3.1. Emulsification procedures

LA and VCA were emulsified in Tween 80% and ethylic alcohol at a proportion of 1:1 (Zhang et al., 2008). This sample represents 1% of the total solution used in the treatments.

### 3.2. Antibacterial activity

This study determined the sensibility of pathogens in response to LA and VCO. To this end, two experiments were conducted in a completely randomized design with five treatments (experiment 1, EXP1: 0, 200, 400, 600 and 800 mg.$L^{-1}$ LA; experiment 2, EXP2: 0, 386.7, 766.0, 1160 and 1500 μL.$L^{-1}$ VCO) and three replicates. Aliquots used in the experiment two have the same concentration at mg.$L^{-1}$ from experiment one.

For the disk-diffusion analysis, a disk of paper (diameter 6 mm) received 10 μL of solution containing the respective concentrations and allocated on a Petri dish containing Brain Heart Infusion Agar (BHI Agar) with *Aeromonas hydrophila*. For the plate diffusion analysis (minimal inhibitory concentration), tubes containing BHI Agar (10 mL at 40 °C) received the cited concentration of each treatment. They were subsequently placed on Petri dishes and inoculated with *Aeromonas hydrophila* at a concentration of $3x10^{10}$CFU.$mL^{-1}$, scattered with the aid of micro pearls.

After 24 h of incubation at 30 °C, each plate was evaluated in terms of its inhibition halo (disk diffusion (Hamal et al., 2008) and expected reduced colony-forming units (CFU) in the plate diffusion (Costa et al., 2017). According to Santurio et al. (2007), the present study considered "biocide concentration" once 100% mortality had occurred.

### 3.3. Antifungal activity

The assay followed the same experimental design and used the same concentrations of antibacterial assay. Disks of agar (9 mm) containing the fungus *Saprolegnia parasitica* were placed at the center of Petri dishes contained the medium and the tested concentrations, and then measured its mycelial growth every 12 h until complete 96 h using a pachymeter (Adapted of Corrêa et al., 2013).

After, we calculated the growth rate index (GRI = diameter – diameter before/number of days) expressed as mm/day (Araújo et al., 2010). As the same in antibacterial assay, the study considered "biocide concentration" once 100% mortality had occurred.

### 3.4. Anti-protozoan activity

This study used the protozoan *Ichthyophthirius multifiliis* at the trophont stage obtained from naturally infested tambaqui (Fu et al., 2014). The experiment occurred in a completely randomized design with five treatments (97.1, 187.39, 289.61, 386.71 and 483.82 μL.$L^{-1}$), representing equal concentrations of LA (0, 50, 150, 200 and 250 mg.$L^{-1}$). When only the LA was being tested, reduced concentrations (0, 10, 20, 30, 40 and 50 mg.$L^{-1}$) were used due to the toxicity. The assay occurred in Petri dishes containing 2 mL of the solutions test with 10 trophonts and three replicates determining mortality and cell division hourly for four hours.

After this, we performed the viability test with a fluorescent probe (SYBR-14 and Propidium iodide IP – molecular probe®) to confirm the real mortality of the parasites. A Nikon Eclipse 50i epifluorescent microscope were used to evaluate the parasite sensibility, which parasites with green color were viable microorganisms, while red color indicated parasites with membrane injuries and could be labeled as dead microorganisms (Nizio et al., 2018).

### 3.5. Statistical analysis

All data were subjected to normality and homoscedasticity tests of Shapiro Wilk and Bartlett respectively. Percentage data received transformation at arc sine square root (x/100) and CFU for log (x + 1). Afterwards, data were conducted to analysis of variance (ANOVA) with post hoc Tukey test ($p < 0.05$) (Zar, 2009).

## 4. Results

### 4.1. Gas chromatographic of fat acids

The coconut oil used in the present study showed the followed composition: caprylic, capric, lauric, myristic, palmitic, stearic, oleic and linoleic acid. Among the several acids, the lauric acid represents 58.7% (Fig. 1).

## 5. In vitro *assay*

### 5.1. Aeromonas hydrophila

The coconut oil and LA had no bactericide effect on *Aeromonas hydrophila* for both methods: inhibition halo or minimal inhibitory concentration . Nonetheless, a reduced number of colonies occurred

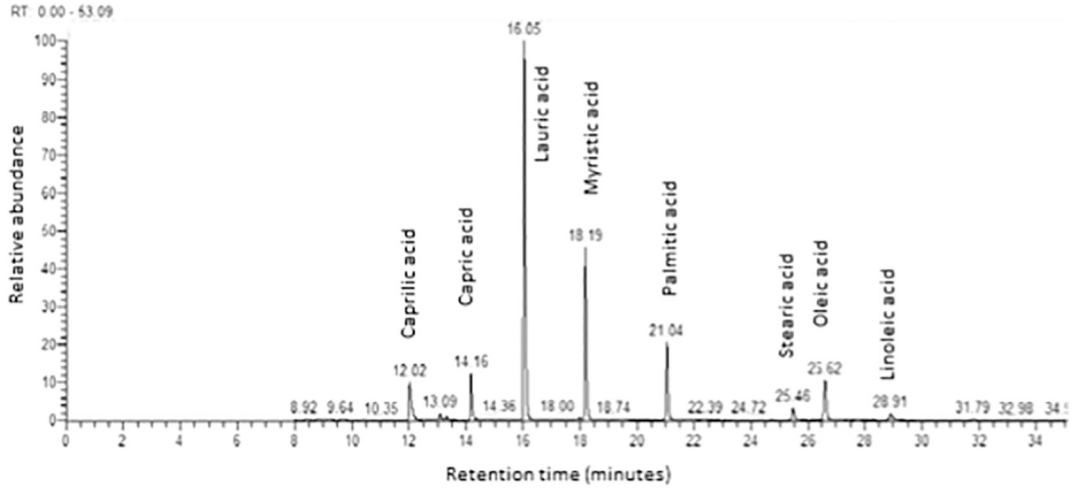


**Fig. 1.** Chromatogram for chemical composition of virgin coconut oil about its fat acid amount.

from the concentrations of 1160.00 μL.L$^{-1}$ coconut oil and 200 mg.L$^{-1}$ LA (Fig. 2).

### 5.2. *Saprolegnia parasitica*

VCO showed reduced mycelial growth at a concentration of 386.7 μL.L$^{-1}$ from the second day (Fig. 3A). However, from the third day, only the concentration 1500 μL.L$^{-1}$ reduced the mycelial growth. On the fourth day, all treatments reached complete the petri dish with mycelial growth. LA provoked reduction on growth at a concentration of 200 mg.L$^{-1}$ and a fungistatic effect at a concentration of 400 mg.L$^{-1}$ from the second day (Fig. 3B).

The concentration of LA showed a negative correlation ($r^2 = 0.94$) considering the mycelial growth, obtaining the followed equation: $y = -0.0479\times + 81.116$ ($p = 0.0047$).

LA and VCO reduced the mycelial growth rate of *Saprolegnia parasitica*. The lowest growth rates for LA and VCO were 766.0 μL.L$^{-1}$ and 200 mg.L$^{-1}$, respectively, from the first day. From the fourth day, mycelial growth complete the petri dish, precluding a growth calculation from being made (Table 1).

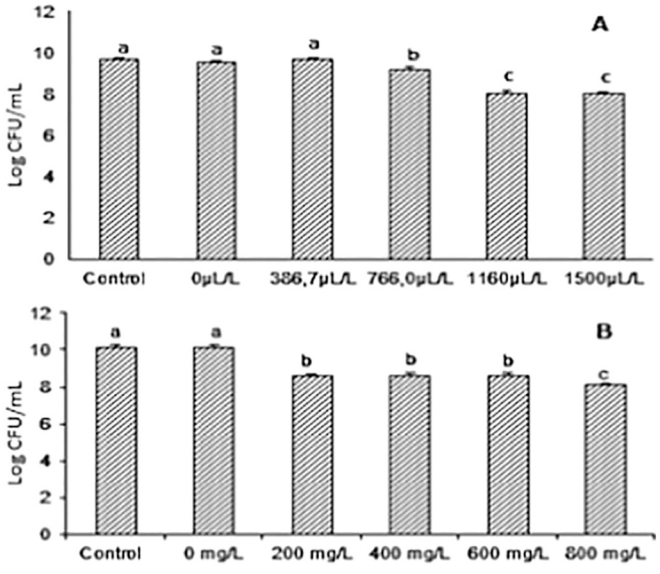


**Fig. 2.** In vitro assay with *Aeromonas hydrophila*: A – Quantification of colonies exposed to different concentrations of virgin coconut oil, B – Quantification of colonies exposed to different concentrations of lauric acid. Different letters on the bars mean statistical difference by Tukey test ($p < 0.05$).

No treatment of VCO caused the real mortality of *Ichthyophthirius multifiliis*, but reduced development of tomonts which occurred after four hours. In addition, greater cell swelling (Fig. 4A) occurred with the 289.61 μL.L$^{-1}$ concentration compared to the control group (Fig. 4B) with formed tomonts. After 24 h, it was possible find the infectious form in all treatments.

Furthermore, with the fourth concentration of LA (40 mg.L$^{-1}$), 100% mortality occurred within the first hour (Fig. 5). By contrast, lower concentrations such as 30 and 20 mg.L$^{-1}$ showed lower mortality of 50 and 20%, respectively. The LA concentrations showed a positive correlation with the parasite mortality rate ($r^2 = 0.91$) as followed equation: $y = 5.2458 + 0.4119$ ($p = 0.0011$).

## 6. Discussion

Eco-friendly alternatives are commonly chosen to control fish diseases, avoiding the selection of more resistant bacteria with chemicals (Harnisz et al., 2015). The VCO stands out as a medium-chain fatty acids with antimicrobial activity (protozoan, bacterium, fungus and virus) (Kappally et al., 2015; Aggarwal et al., 2017).

In this study, VCO showed a direct effect on fungal growth (*Saprolegnia parasitica*), similar to various fungi that cause problems to plants (Walters et al., 2003), food decomposition and fungi of human as *Candida* sp. Tjin et al. (2016) found higher concentrations of VCO (2 g equivalent to LA 1 g) to control *Candida albicans*, with fungistatic action from the second day. The present study found a similar result with LA to control *Saprolegnia parasitica*.

Beyond their fungicidal effects, VCO and LA can change the structure and pathogenic mechanisms provoking reduced virulence (Liang et al., 2018). This effect can be considered beneficial, reducing the virulence of *Saprolegnia parasitica* and avoiding the outbreak of diseases in eggs or fish (Lone and Manohar, 2018).

The same effect was observed to the parasite pathogen, both VCO and LA affected the development of *Ichthyophthirius multifiliis*, but only the latter caused the mortality of the parasite. The effect of LA has previously been reported for the *Giardia duodenalis* at a concentration of 100 mg.L$^{-1}$ (Rayan et al., 2005), higher than concentrationused in the present study.

According to Rayan et al. (2005), LA can penetrate cells and cause cytoplasmic alterations, changing the permeability of the membrane and provoking it to break up. The same mechanism probably occurred with the *Ichthyophthirius multifiliis* in this study, but detailed analysis remains necessary to confirm this finding. By contrast, VCO did not cause mortality, probably because fatty acids did not penetrate the

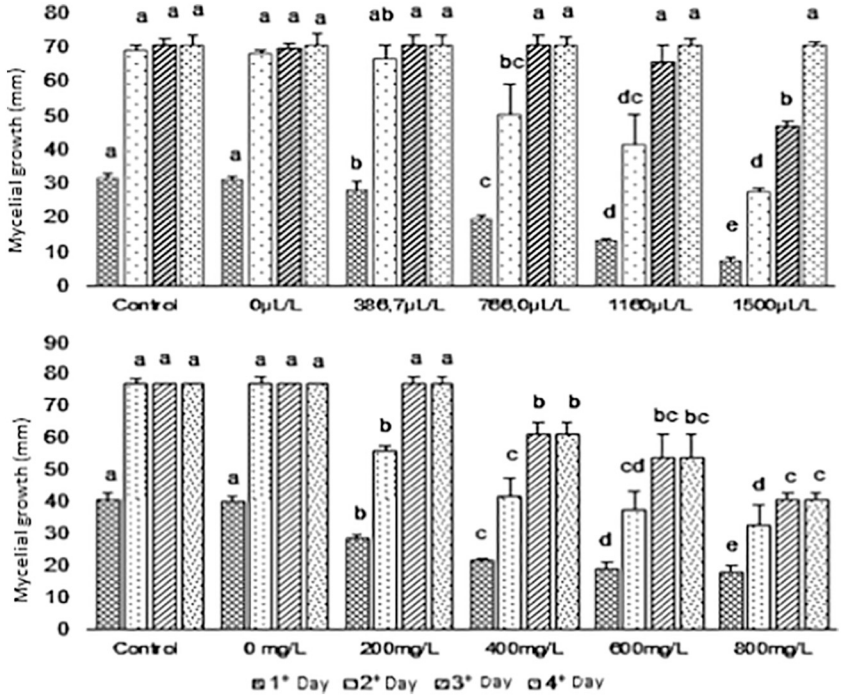


**Fig. 3.** Micelial growth (mm) in vitro assay for *Saprolegnia parasitica*: A – Experiment with coconut oil*, B – Experiment with lauric acid, *the Coconut oil concentration is equivalent to lauric acid concentrations.

**Table 1**
Micelial growth rate indexes (mm.day$^{-1}$) of *Saprolegnia parasitica* exposed to different concentrations of lauric acid and virgin coconut oil.

| Concentration | 1° Day | 2° Day | 3° Day | 4° Day |
|---|---|---|---|---|
| Virgin coconut oil* | | | | |
| Control | 30.25 ± 1.52Aa | 19.08 ± 0.76Ba | 0.37 ± 0.06Cb | 0.00 ± 0.00Dc |
| 0.0 µL.L$^{-1}$ | 29.80 ± 1.70Aa | 19.80 ± 1.90Ba | 0.37 ± 0.06Cb | 0.00 ± 0.00Dc |
| 386.7 µL.L$^{-1}$ | 27.03 ± 2.20Aa | 19.21 ± 1.55Ba | 1.35 ± 1.03Cb | 0.00 ± 0.00Dc |
| 766.0 µL.L$^{-1}$ | 18.61 ± 1.70Ab | 15.29 ± 4.45Aab | 6.77 ± 2.71Ba | 0.00 ± 0.00Cc |
| 1160 µL.L$^{-1}$ | 12.12 ± 1.65Ac | 14.17 ± 2.04Abc | 7.93 ± 1.91Ba | 1.74 ± 1.02Cb |
| 1500 µL.L$^{-1}$ | 6.02 ± 0.90 Bd | 10.30 ± 0.81Ac | 6.41 ± 0.67Ba | 6.95 ± 2.67Ba |
| Lauric acid (Sigma > 98%) | | | | |
| Control | 40.71 ± 1.87Aa | 19.62 ± 0.31Ba | 18.14 ± 0.94Ba | 0.00 ± 0.00Ca |
| 0 mg.L$^{-1}$ | 39.90 ± 2.07Aa | 20.30 ± 0.67Ba | 18.95 ± 1.02Ba | 0.00 ± 0.00Ca |
| 200 mg.L$^{-1}$ | 28.27 ± 0.88Ab | 21.10 ± 0.17Ba | 13.69 ± 0.50Cb | 0.00 ± 0.00 Da |
| 400 mg.L$^{-1}$ | 21.29 ± 0.79Ac | 16.99 ± 1.14Bb | 10.26 ± 2.89Cbc | 0.00 ± 0.00 Da |
| 600 mg.L$^{-1}$ | 19.07 ± 2.00Ad | 14.88 ± 2.15ABb | 9.20 ± 2.73Bc | 0.00 ± 0.00Ca |
| 800 mg.L$^{-1}$ | 18.08 ± 2.09Ad | 15.54 ± 2.09Ab | 7.22 ± 3.60Bc | 0.00 ± 0.00Ca |

Different letters in the column means statistical difference by Tukey test ($p < 0.05$). *Concentrations equals to the lauric acid based on gas chromatographic analysis.

membrane, so rather than the membrane breaking up, only swelling occurred (Hilder, 1968; Wang and Johnson, 1992).

In this study, *Aeromonas hydrophila* showed resistance to VCO and LA, in contrast to Nitbani et al. (2016), who identified the antimicrobial potential of LA at 150 mg.L$^{-1}$ against *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*, with higher inhibition halos than Ciprofloxacin.

VCO and LA reduced the inhibitory effect against Gram-negative bacteria relative to their Gram-positive counterparts (Abbas et al., 2017). Despite their lack of bactericidal effect, they can affect bacteria's development, probably due to the viscosity of the oil interfering with the adhesion of bacteria on Petri dishes (Kaushik et al., 2016) being a physical and not a biological effect.

The contrasting results regarding the use of VCO and LA to control fish pathogens may be related to some inhibitory effect of the former on the latter, as observed by Nitbani et al. (2016) and Nagase et al. (2017). However, further studies are required to evaluate this finding.

The results of this study have demonstrated a possible strategy for controlling *Ichthyophthirius multifiliis* and *Saprolegnia parasitica*: the use of therapeutic baths. Nevertheless, their antimicrobial effects may increase when LA is converted into monolaurin. The latter has been identified as having considerable antimicrobial potential, degrading the beta-glucan on the microorganism membrane, breaking it up, and causing reduced cell breathing (Bergsson et al., 2001; Dayrit, 2015; Luo et al., 2014). These mechanisms caused the mortality of *Candida albicans* and *Giardia lamblia* in this study (Rayan et al., 2005; Seleem et al., 2016).

However, the natural change of lauric acid for monolaurin only occurs into digestive tract. That specific characteristic can suggest for adequate control of *Aeromonas hydrophila, Ichthyophthirius multifiliis* and *Saprolegnia parasitica*, would be better incorporate the VCO on feed to increase the positive results or combined with therapeutic baths.

## 7. Conclusion

Virgin coconut oil can affect the development of microorganisms, but the lauric acid has fungistatic effect, reduces the bacterial growth of *Aeromonas hydrophila* and cause mortality of *Ichthyophthirius multifiliis*. Nonetheless, more studies must be carried out to determine the real mechanism of action against fish pathogens.

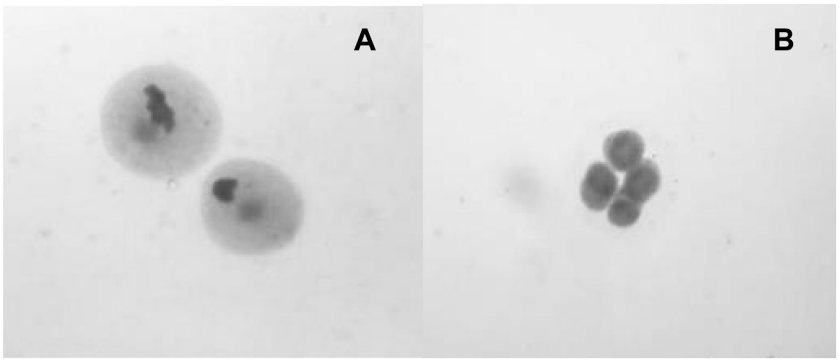


**Fig. 4.** In vitro assay with *Ichthyophthirius multifiliis* submitted to different concentrations of virgin coconut oil throughout the four hours. A – Trophont at treatment with virgin coconut oil, B – Control without virgin coconut oil at tomont stage.

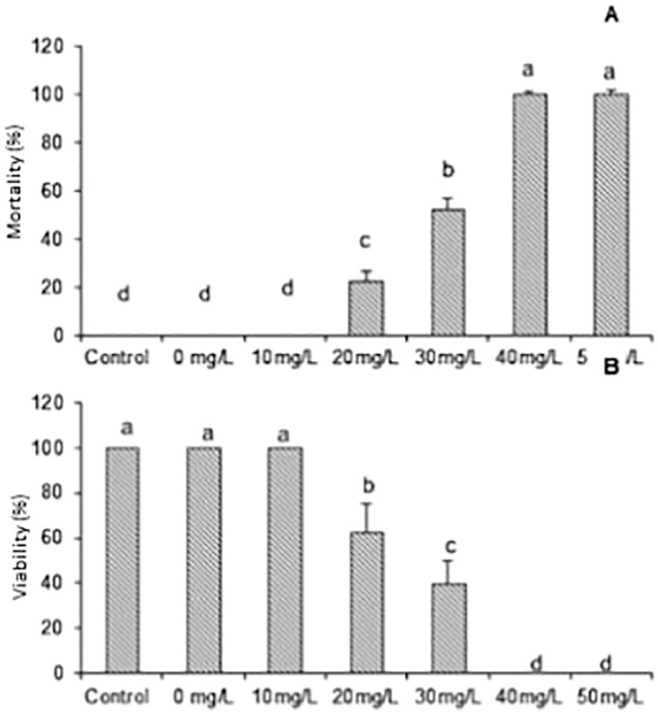


**Fig. 5.** In vitro assay with *Ichthyophthirius multifiliis* submitted to different concentrations of lauric acid in four hours of exposure. A – mortality rate of protozoan, B – viability test with one hour of experiment, Different letters on the bars mean statistical difference by Tukey test ($p < 0.05$).

## Data availability statement

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

## Funding statement

There is no funding for this study.

## Declaration of Competing Interest

The authors have no any conflict of interest to declare.

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