BOLETIM DO INSTITUTO DE PESCA
ISSN 1678-2305 online version
Scientific Article

TOXICITY OF *Lippia origanoides* ESSENTIAL OIL IN TAMBAQUI (*Colossoma macropomum*) AND ITS EFFECT AGAINST *Aeromonas hydrophila**

Susanne Regina Nazaré de OLIVEIRA[1]
Marjorie Aymê Souza de OLIVEIRA[2]
Franmir Rodrigues BRANDÃO[3]
Cláudia MAJOLO[4]
Francisco Célio Maia CHAVES[4]
Edsandra Campos CHAGAS[3,4]

[1]Uninorte Laureate International Universities, Av. Joaquim Nabuco 1469, Centro, CEP 69020-030, Manaus, AM, Brasil.

[2]Universidade Federal do Amazonas - UFAM, Av. General Rodrigo, Octávio 620, Coroado I, CEP 69077-000, Manaus, AM, Brasil

[3]Universidade Federal do Amazonas – UFAM, Programa de Pós-graduação em Ciências Pesqueiras nos Trópicos, Av. General Rodrigo, Octávio 620, Coroado I, CEP 69077-000, Manaus, AM, Brasil.

[4]Embrapa Amazônia Ocidental, AM-010, CP 319, CEP 69010-970, Manaus, AM, Brasil. E-mail: edsandra.chagas@embrapa.br (corresponding author)

*Financial support: Embrapa (MP2 - 02.12.01.020.00.00) and FINEP (#DARPA project 01.09.0472.00).

Received: December 15, 2017
Approved: February 14, 2018

ABSTRACT

The study evaluated the toxicity of *Lippia origanoides* essential oil (EO) in tambaqui (*Colossoma macropomum*) and its *in vivo* activity against *Aeromonas hydrophila*. Toxicity was tested by tambaqui exposure to 0, 5, 10, 12.5, 15, 17.5 and 20 mg L^{-1} of *L. origanoides* EO for 96 h. The mean lethal concentration (LC_{50}) was 15.2 mg L^{-1}. After tambaqui exposure to sublethal concentrations of *L. origanoides* EO (7.6 and 11.4 mg L^{-1}) for 96 h, only the 7.6 mg L^{-1} level caused minor hematologic and biochemical changes that include increase in mean corpuscular volume and decrease in total protein, which did not compromise fish homeostasis. Tambaqui experimentally infected with *A. hydrophila* had higher survival rate (79.2%) after therapeutic bath with 10 mg L^{-1} *L. origanoides* EO. Therefore, tambaqui exhibits moderate tolerance to *L. origanoides* EO, and this oil has potential for use in the treatment of tambaqui infected with *A. hydrophila*.

Key words: fish; hematology; LC_{50}; pathogenic bacteria; survival.

TOXICIDADE DO ÓLEO ESSENCIAL DE *Lippia origanoides* EM TAMBAQUI (*Colossoma macropomum*) E SEU EFEITO FRENTE À *Aeromonas hydrophila*

RESUMO

O presente estudo avaliou a toxicidade do óleo essencial de *Lippia origanoides* em tambaqui (*Colossoma macropomum*) e sua atividade *in vivo* frente à *Aeromonas hydrophila*. Para os testes de toxicidade, tambaquis foram expostos a 0, 5, 10, 12,5, 15, 17,5 e 20 mg L^{-1} do óleo essencial de *L. origanoides* por 96 horas. A concentração média letal (CL_{50}) foi estimada em 15,2 mg L^{-1}. Após exposição dos tambaquis às concentrações subletais de *L. origanoides* (7,6 e 11,4 mg L^{-1}) por 96 horas, poucas alterações hematológicas e bioquímicas como aumento no volume corpuscular médio e decréscimo nos valores de proteínas totais foram observadas na concentração de 7,6 mg L^{-1}, o que não compromete a homeostase orgânica dos peixes. Tambaquis experimentalmente infectados com *A. hydrophila* alcançaram maior taxa de sobrevivência (79,2%) após banho terapêutico com 10 mg L^{-1} do óleo essencial de *L. origanoides*. Portanto, o tambaqui exibe moderada tolerância ao óleo essencial de *L. origanoides* e este óleo tem potencial para uso no tratamento de tambaqui (*C. macropomum*) infectado com *A. hydrophila*.

Palavras-chave: peixe; hematologia; CL_{50}; bactéria patogênica; sobrevivência.

INTRODUCTION

Tambaqui (*Colossoma macropomum*) is the main native fish species farmed in Brazil with a production of 137 thousand metric tons in 2016 (IBGE, 2016). In intensive production, its body weight reaches 2.62 kg, and after 10 months of growing in dugout ponds, it can yield 18,530 kg ha^{-1} (IZEL *et al.*, 2013). In several regions of the country, however, the growth and intensification of fish farming systems are commonly accompanied by inadequate management practices and outbreaks of parasitic and bacterial diseases (CHAGAS *et al.*, 2015; VALLADÃO *et al.*, 2016; TAVARES-DIAS and MARTINS, 2017).

In aquaculture, bacterial diseases account for economic losses worldwide (AUSTIN and AUSTIN, 2007). In Brazilian fish farms, such outbreaks are frequently recorded

(SEBASTIÃO *et al*., 2015; VALLADÃO *et al*., 2016; TAVARES-DIAS and MARTINS, 2017) and are mainly associated with the bacteria *Aeromonas hydrophila*, *Flavobacterium columnare* and *Streptococcus agalactiae*, which cause hemorrhagic septicemia, columnaris and streptococcosis, respectively (FIGUEIREDO and LEAL, 2008; PILARSKI *et al*., 2008; SEBASTIÃO *et al*., 2015).

Antibiotics and other drugs are used to treat bacterial diseases in aquaculture, but their indiscriminate application has caused environmental pollution, residue accumulation in the muscle of commercialized fish and the development of drug-resistant bacterial strains (CHAKRABORTY and HANCZ, 2011). For instance, *A. hydrophila* isolated from pacu (*Piaractus mesopotamicus*) and Nile tilapia (*Oreochromis niloticus*) in Brazil were found to be resistant to antibiotics (BELÉM-COSTA and CYRINO, 2006). Thus, essential oils (EO) and bioactive plant extracts have been increasingly investigated as alternative therapeutic products for aquaculture (REVERTER *et al*., 2014; HASHIMOTO *et al*., 2016; SILVA *et al*., 2017; BRUM *et al*., 2017).

Studies on the control of bacterial diseases by natural products show promising results (ZHENG *et al*., 2009; ALSAID *et al*., 2010; MEEPAGALA *et al*., 2013). In Nile tilapia, *Cratoxylum formosum* extract was found to control *S. agalactiae*, and therapeutic baths with *Centella asiatica* extract efficiently reduced mortality of fish infected with *F. columnare*, in a dose-dependent response (RATTANACHAIKUNSOPON and PHUMKHACHORN, 2010a,b). In addition, *Lippia alba* EO increased survival time of jundiá (*Rhamdia quelen*) after the challenge test with *A. hydrophila* (SUTILI *et al*., 2015a).

Different *Lippia* species have been tested in aquaculture due to the antimicrobial potential of this genus (SUTILI *et al*., 2015a; MAJOLO *et al*., 2017; SOUZA *et al*., 2017a). One important species is *Lippia origanoides*, native of Central and South America and popularly known as *salva-de-Marajó* (LORENZI and MATOS, 2008). Its EO contains carvacrol, thymol and γ-terpinene, which show antimicrobial activity against *Lactobacillus casei*, *Streptococcus mutans*, *Salmonella enteritidis*, *Escherichia coli* and *A. hydrophila* (OLIVEIRA *et al*., 2007; HENAO *et al*., 2010; BETANCOURT *et al*., 2012; MAJOLO *et al*., 2017).

The present study evaluated the toxicity of *L. origanoides* EO in tambaqui and its *in vivo* activity against *A. hydrophila*.

METHODS

Plant species, extraction and chemical characterization of the essential oil

Voucher specimens of the *L. origanoides* (family Verbenaceae) plants were deposited in the IAN Herbarium, at Embrapa Amazônia Oriental, Belém, Pará state (PA) (No. 191734). The plants were cropped in the Medical Plants and Vegetables Division of Embrapa Amazônia Ocidental, Manaus, Amazonas state (AM). The branches were cut and the leaves separated and dried in the shadow until reaching constant weight. In the Laboratory of Medical Plants and Phytochemistry, essential oil (EO) from the dried leaves was extracted by hydro distillation for 2 h in a Clevenger-type apparatus. The EO was stored frozen in amber flasks, at -4 °C. The chemical composition of *L. origanoides* EO was determined by gas chromatography and mass spectrometry at Embrapa Agroindústria de Alimentos, according to POTZERNHEIM *et al*. (2012).

Fish acclimatization

The juvenile tambaqui were obtained from the Santo Antônio Farm (Rio Preto da Eva, AM) and transported to the experimental field at Embrapa Amazônia Ocidental (Manaus, AM), where they were held in 200 m^2 ponds. An YSI Pro20 dissolved oxygen meter and YSI F-1100 digital pH meter were used to measure water parameters three times a week. On average, these were kept at a temperature of 29.19 ± 0.29 °C, dissolved oxygen of 5.82 ± 0.22 mg L^{-1} and pH of 6.32 ± 0.07. Alkalinity (17.4 ± 2.88 mg L^{-1}) and total ammonia (0.98 ± 0.10 mg L^{-1}) were measured every 15 days using EDTA and indophenol titration, respectively (APHA, 1998). In this period, fish were fed commercial food for omnivorous fish containing 32% crude protein (CP).

Acute toxicity (96-h LC_{50}) of *Lippia origanoides* EO

In the assays determining mean lethal concentration (LC_{50}) of *L. origanoides* EO, the tambaqui (n = 168; 139.33 ± 5.52 g and 19.94 ± 0.35 cm) were transferred to 80-L polyethylene tanks supplied with a static constant aeration system. After adjusting to the conditions for 48 h, fish were exposed to *L. origanoides* EO at 0, 5, 10, 12.5, 15, 17.5 and 20 mg L^{-1} concentrations for 96 h, in triplicate.

Mortality rate and feed intake were recorded and fish behavior was observed 24, 48, 72 and 96 h after *L. origanoides* EO was introduced into the water, twice a day. The behaviors recorded included loss of balance, opercular movement and erratic swimming. Fish were considered dead when resting motionless on the bottom of the tank, with no operculum movement and unresponsive to mechanical stimulation.

Water quality was monitored during the tests, showing a temperature of 28.7 ± 0.5 °C, dissolved oxygen of 6.3 ± 0.3 mg L^{-1}, pH of 6.65 ± 1.41, alkalinity of 16.02 ± 0.46 mg L^{-1} and total ammonia content of 0.41 ± 0.04 mg L^{-1}.

Sublethal effects of *Lippia origanoides* EO on tambaqui

After 96-h LC_{50} was determined, another batch of tambaqui (n = 72, 149.44 ± 7.38 g and 21.37 ± 0.33 cm) were exposed to sublethal concentrations of *L. origanoides* EO for the same period. The treatments, performed in triplicate, consisted of exposure to 0%, 50% and 75% of the 96-h LC_{50}, that is, 0 (control), 7.62 and 11.43 mg L^{-1} EO, respectively.

To evaluate hematologic and biochemical parameters, after 96 h exposure to the treatments, fish were anesthetized with 100 mg L^{-1} of benzocaine and their blood collected by caudal vein puncture with heparinized syringes.

The hematologic parameters determined were hematocrit (HCT), hemoglobin (Hb) and red blood cells (RBC). For HCT determination, blood samples were centrifuged in heparinized capillary tubes (15,000 g for 10 min) before being read on a standardized scale. Hb was determined by the cyanmethemoglobin method, and RBC count was performed in a Neubauer chamber after blood dilution in formalin citrate fluid. These parameters were used to calculate mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) (BROW, 1988). Commercial kits (Labtest®) were used to determine plasma glucose applying the glucose oxidase method and total protein by the biuret method.

Water quality was monitored during the tests and kept at a temperature of 28.36 ± 0.84 °C, dissolved oxygen of 6.71 ± 0.60 mg L^{-1}, pH of 6.24 ± 0.80, alkalinity of 17.76 ± 0.41 mg L^{-1} and total ammonia content of 0.50 ± 0.07 mg L^{-1}.

Effect of *Lippia origanoides* EO on survival of tambaqui infected with *Aeromonas hydrophila*

The juvenile tambaqui (n = 96, 90.56 ± 5.54 g and 18.01 ± 0.36 cm) were held in twelve 1000-L fiberglass tanks (12 fish per tank) supplied with water recirculation and constant aeration. Fish were allowed to adapt to tank conditions for 30 days, fed a diet containing 32% CP twice a day, to apparent satiety.

A completely randomized design was used with 4 treatments and 3 replicates. The treatments were: 1) control (fish inoculated with *A. hydrophila* and not treated with EO), 2) antibiotic treatment (10 mg L^{-1} chloramphenicol), 3) EO treatment 1 (5 mg L^{-1} *L. origanoides*), and 4) EO treatment 2 (10 mg L^{-1} *L. origanoides*). To that end, fish were collected, anesthetized with 100 mg L^{-1} of benzocaine and inoculated with 1.0 x 10^8 CFU *A. hydrophila*. They were then transferred to 80-L tanks supplied with a static constant aeration system, and the therapeutic bath (specific for each treatment) was applied for 60-min and repeated for 5 consecutive days. Fish were then returned to 1000-L fiberglass tanks (SUTILI *et al*., 2015a,b). Fish mortality was assessed every 24 h for 10 days.

Water quality was monitored during the tests, showing a temperature of 29.62 ± 0.04 °C, dissolved oxygen of 6.97 ± 0.03 mg L^{-1}, pH of 7.28 ± 0.06, alkalinity of 17.22 ± 0.44 mg L^{-1} and total ammonia content of 0.45 ± 0.04 mg L^{-1}.

Statistical analysis

The results obtained are expressed as mean ± standard error. LC_{50} was calculated using the Trimmed Spearman Karber method (HAMILTON *et al*., 1977). The homogeneity of variances of data was tested by Levene Test and the data normality assessed by the Shapiro-Wilk test. Data on response to sublethal EO exposure and survival (data transformed) were compared by analysis of variance (one-way ANOVA), followed by a post hoc test (Tukey and Duncan, respectively) ($P < 0.05$).

RESULTS

Eighteen compounds were identified in the essential oil of *L. origanoides*, comprising 92.7% of the oil composition. Quantitatively, the most abundant were carvacrol (49.7%), para-cymene (13.3%) and thymol (9.9%) (Table 1).

Mortalities were not observed after 96 h exposure to the treatments with 0, 5 and 10 mg L^{-1}. On the other hand, no fish survived using a concentration of 20 mg L^{-1} EO (Figure 1). The 96-h LC_{50} of *L. origanoides* EO for tambaqui, with a 95% confidence interval, is shown in Figure 1.

Table 1. Chemical composition of *Lippia origanoides* essential oil.

Components	*L. origanoides (%)*	RI*
(E)-2-hexenal	0.5	853
α-thujene	1.2	928
α-pinene	0.5	936
1-octen-3-ol	0.6	977
myrcene	2.4	989
α-terpinene	1.1	1016
para-cymene	13.3	1025
1,8-cineole	0.9	1032
γ-terpinene	4.5	1059
linalool	2.8	1096
ipsdienol	0.4	1144
umbelulone	1.1	1175
thymol methyl ether	0.9	1232
thymol	9.9	1288
carvacrol	49.7	1298
carvacrol acetate	0.4	1369
(E)-β-caryophyllene	1.5	1414
caryophyllene oxide	1.0	1576
Total identified compounds	92.7	-

*Retention Index.

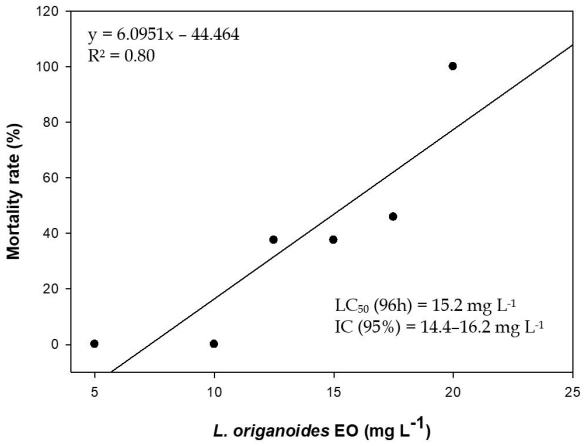

Figure 1. Mortality rate 96h after exposure of juvenile tambaqui (*Colossoma macropomum*) to different concentrations of *Lippia origanoides* essential oil.

In the acute toxicity evaluation, the EO concentrations tested (except for the control treatment) promote some behavioral alterations such as accelerated opercular movement and mucus secretion 24h after exposure. These responses were more pronounced in treatments with 17.5 and 20 mg L^{-1} EO.

Tambaqui exposure to the two sublethal concentrations of *L. origanoides* EO (7.6 and 11.4 mg L^{-1}) for 96 h did not change HCT, Hb, RBC, MCHC or plasma glucose, but at 7.6 mg L^{-1} it increased MCV and decreased total protein in relation to the control group (Table 2).

After the therapeutic bath assay, treatments with EO were statistically different between them, but they did not differ from the control group or those treated with antibiotics (Figure 2).

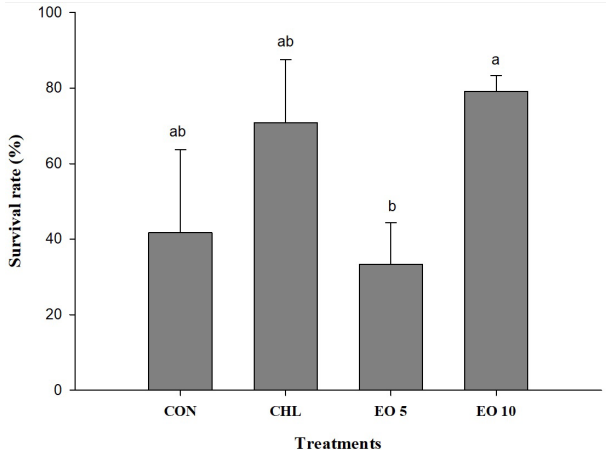

Figure 2. Survival rate of tambaqui infected with *Aeromonas hydrophila* and subjected to therapeutic baths with *Lippia origanoides* essential oil. Bars with different letters indicate significant differences between the treatments as determined by one-way ANOVA and Duncan´s test (P<0.05). CON = control treatment, without oil or antibiotic; CHL = antibiotic treatment with 10 mg L^{-1} chloramphenicol; EO5 and EO10 = treatment with 5 and 10 mg L^{-1} *L. origanoides* EO, respectively.

DISCUSSION

The major compounds of *L. origanoides* EO used in this study were carvacrol (49.7%), para-cymene (13.3%) and thymol (9.9%). These compounds were also found in the EO of *L. origanoides* collected from the Chicamocha river canyon (Santander, Colombia) by VICUNA *et al*. (2010), but in this study thymol (34–58%) presented a higher relative amount than that observed in the present study, suggesting that these variations can be influenced by differences in climatic conditions, geographical origin, seasonality, stage of plant development, procedures adopted for plant processing and oil extraction, and plant chemotype (SANTOS *et al*., 2004; MARCIAL *et al*., 2016).

Few studies report the toxicity of essential oils to fish (YAO *et al*., 2011; ABD EL-GALIL and ABOELHADID, 2012; KUMAR *et al*., 2012). To *L. origanoides* EO this information is scarce. To *Chenopodium abrosioides* aqueous extract the 24-h LC_{50} for tambaqui is 2.6 ml L^{-1} (MONTEIRO, 2012), and the 4h-LC_{50} of *Mentha piperita* EO for pirarucu (*Arapaima gigas*) is 38 mg L^{-1} (MALHEIROS *et al*., 2016). Compared to these studies, tambaqui was more tolerant to *L. origanoides* EO (LC_{50} 15.24 mg L^{-1}), especially because the evaluation period was longer (96 h). The results obtained in this study with tambaqui are important given the scarcity of studies investigating the toxicity of *L. origanoides* EO or its main components, which are carvacrol, p-cymene and thymol.

In the toxicity test to tambaqui was observed an accelerated opercular movement and mucus secretion after exposure to EO *L. origanoides*, being more pronounced at higher EO concentrations (17.5 and 20 mg L^{-1}). For other *Lippia* species was observed that tambaqui treated with 30-min baths containing *L. alba* EO at higher concentrations (100 and 150 mg L^{-1}) showed an increase in opercular beat rate and mucus secretion along with severe gill lesions, such as hyperplasia and lamellar epithelial fusion, congestion, edema and necrosis, proliferation of mucous and chloride cells and lamellar hypertrophy (SOARES *et al*., 2016).

Tambaqui exposure at a concentration of 7.6 mg L^{-1} of *L. origanoides* EO increased MCV and decreased total protein in relation to the control group. These results are different to

Table 2. Hematologic parameters of tambaqui, *Colossoma macropomum*, after 96-h exposure to sublethal concentrations of *Lippia origanoides* essential oil (EO).

Parameters	*Lippia origanoides* EO concentration (mg L^{-1})		
	0	7.6	11.4
HCT (%)	26.21 ± 0.78^{a}	27.92 ± 0.90^{a}	28.20 ± 0.64^{a}
Hb (g dL^{-1})	7.82 ± 0.22^{a}	7.80 ± 0.30^{a}	7.92 ± 0.20^{a}
RBC (10^{6} mm^{-3})	1.60 ± 0.05^{a}	1.54 ± 0.05^{a}	1.68 ± 0.05^{a}
MCV (μm^{3})	164.37 ± 3.92^{a}	181.57 ± 5.05^{b}	168.17 ± 4.25^{ab}
MCH (pg)	48.97 ± 0.75^{a}	50.67 ± 1.71^{a}	47.54 ± 1.88^{a}
MCHC (%)	29.99 ± 0.87^{a}	28.02 ± 0.91^{a}	28.15 ± 1.00^{a}
Glucose (mg dL^{-1})	88.67 ± 9.88^{a}	63.67 ± 8.49^{a}	71.92 ± 6.80^{a}
Protein (g dL^{-1})	3.23 ± 0.13^{a}	2.73 ± 0.10^{b}	2.92 ± 0.10^{ab}

Different letters indicate significant differences between the treatments as determined by one-way ANOVA and Tukey´s test (P<0.05). Abbreviations: HCT = hematocrit, Hb = hemoglobin, RBC = red blood cell, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration.

those of SOARES *et al*. (2017a) who related an increased in plasma protein concentration in tambaqui exposed to 20 and 40 mg L^{-1} of *L. origanoides* essential oil for 60 and 30 minutes, respectively. For other *Lippia* species, increased MCV was observed in tambaqui exposed to 20 mg L^{-1} of *L. sidoides* for 15 minutes because of a decrease in the number of erythrocytes, hematocrit and hemoglobin (SOARES *et al*., 2017b). Considering plasma glucose levels, no significant alterations were observed between treatments with *L. origanoides* EO, probably due to the anaesthetic effect of this oil, as observed in others studies with tambaqui during the therapeutic baths with *L. origanoides* EO (SOARES *et al*., 2017a). Similar pattern was observed by SENA *et al*. (2016) using *L. alba* EO to reduce effects of stress in tambacu (*P. mesopotamicus* x *C. macropomum*). Therefore, tambaqui subjected to sublethal concentrations of the *L. origanoides* EO did not exhibit significant physiological responses that indicate homeostatic disturbance, as observed in others studies with others *Lippia* species, since the hematologic and biochemical parameters tested in tambaqui were within the normal range for healthy fish (TAVARES-DIAS, 2015). Thus, the minor physiological changes observed do not characterize homeostatic imbalance and do not preclude the use of *L. origanoides* EO to treat bacterial and parasitic diseases.

Survival of tambaqui treated with *L. origanoides* EO was above 30% and around 80% using 5 and 10 mg L^{-1} oil. Similar results were found in studies on silver catfish (*Rhamdia quelen*) infected with *A. hydrophila* and treated with different natural products in therapeutic bath protocols. Silver catfish survival was nearly 80% in fish treated with 16 and 40 mg L^{-1} *L. alba* EO (SUTILI *et al*., 2015a), 70 and 66% with 20 and 40 mg L^{-1} *Hesperozygis ringens* EO, respectively, 75% with 10 mg L^{-1} *Ocimum americanum* EO (SUTILI *et al*., 2015b), and 37 and 66% with 5 and 10 mg L^{-1} eugenol oil, respectively (SUTILI *et al*., 2014).

A noteworthy point is that thymol and carvacrol are among the main components of *L. origanoides* EO. These compounds are found in different *Lippia* species and account for the inhibitory activity against *A. hydrophila* (MAJOLO *et al*., 2017). This occurs because these phenolic terpenes exhibit strong antimicrobial activity, which can disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides and increasing cytoplasmic membrane permeability to ATP (HELANDER *et al*., 2001).

Increased survival of channel catfish (*Ictalurus punctatus*) challenged with *A. hydrophila* was obtained with compounds carvacrol and thymol, the main components of *L. origanoides* EO, but in protocol of diet supplementation (ZHENG *et al*., 2009). Similar results were obtained in Nile tilapia treated with green tea (*Camellia sinensis*) (ABDEL-TAWWAB *et al*., 2010), rainbow trout (*Oncorhynchus mykiss*) treated with methanolic nettle root extracts (*Urtica dioica*) (BILEN *et al*., 2016) and rohu (*Labeo rohita*) treated with aqueous extract of *Ocimum sanctum* (DAS *et al*., 2015). Therefore, some EO and extract of bioactive plants are effective in promotes survival of fish in different administration protocols. However, further studies should be carried out to improve the therapeutic efficacy of *L. origanoides* EO against *A. hydrophila* in tambaqui as the use of nanoemulsions (THOMAS *et al*., 2013) and nanotechnology (SOUZA *et al*., 2017b).

CONCLUSIONS

Tambaqui exhibit moderate tolerance to *L. origanoides* EO, showing 96-h LC_{50} of 15.2 mg L^{-1}. In addition, sublethal concentrations caused only a few hematologic and biochemical changes that do not characterize homeostatic imbalance. *L. origanoides* EO shows potential for use in the treatment of tambaqui infected with *A. hydrophila*.

ACKNOWLEDGEMENTS

To Dr. Marcelo Róseo de Oliveira for helping with essential oil extraction and the laboratory assistant Iraní Morais for assisting in physiological parameter analysis.

REFERENCES

ABD EL-GALIL, M.A.; ABOELHADID, S.M. 2012 Trials for the control of trichodinosis and gyrodactylosis in hatchery reared *Oreochromis niloticus* fries by using garlic. *Veterinary Parasitology*, *185*(2-4): 57-63. **http://dx.doi.org/10.1016/j.vetpar.2011.10.035**. PMid:22137346.

ABDEL-TAWWAB, M.; AHMAD, M.H.; SEDEN, M.E.A.; SAKR, S.F.M. 2010 Use of green tea, *Camellia sinensis* L. in practical diet for growth and protection of Nile tilapia, *Oreochromis niloticus* (L.) against *Aeromonas hydrophila* infection. *Journal of the World Aquaculture Society*, *41*(s2): 203-213. **http://dx.doi.org/10.1111/j.1749-7345.2010.00360.x**.

ALSAID, M.; DAUD, H.; BEJO, S.K.; ABUSELIANA, A. 2010 Antimicrobial activities of some culinary spice extracts against *Streptococcus agalactiae* and its prophylactis uses to prevent streptococcal infection in red hybrid tilapia (*Oreochromis* sp.). *World Journal of Fish Marine Sciences*, *2*(6): 532-538.

APHA – AMERICAN PUBLIC HEALTH ASSOCIATION, 1998 *Standard Methods for the Examination of Water and Wastewater*. 20th ed. Washington: APHA. 937 p.

AUSTIN, B.; AUSTIN, D.A. 2007 *Bacterial fish pathogens*. Springer-Science, Chichester. UK. 552 p.

BELÉM-COSTA, A.; CYRINO, J.E.P. 2006 Antibiotic resistence of *Aeromonas hydrophila* isolated from *Piaractus mesopotamicus* (Holmberg, 1887) and *Oreochromis niloticus* (Linnaeus, 1758). *Scientia Agrícola*, *63*(3): 281-284. **http://dx.doi.org/10.1590/S0103-90162006000300011**.

BETANCOURT, L.; PHANDANAUVONG, V.; PATIÑO, R.; ARIZA-NIETO, C.; AFANADOR-TELLEZ, G. 2012 Composition and bactericidal activity against beneficial and pathogenic bacteria of oregano essential oils from four chemotypes of *Origanum* and *Lippia* genus. *Revista de la Facultad de Medicina Veterinaria y Zootecnia*, *59*(1): 21-31.

BILEN, S.; ÜNAL, S.; GÜVENSOY, H. 2016 Effects of oyster mushroom (*Pleurotus ostreatus*) and nettle (*Urtica dioica*) methanolic extracts on imune responses and resistance to *Aeromonas hydrophila* in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *454*: 90-94. **http://dx.doi.org/10.1016/j.aquaculture.2015.12.010**.

BROW, B.A. 1988 *Hematology: principles and procedures*. 5th ed. Philadelphia: Lea & Febiger. 461 p.

BRUM, A.; PEREIRA, S.A.; OWATARI, M.S.; CHAGAS, E.C.; CHAVES, F.C.M.; MOURIÑO, J.L.P.; MARTINS, M.L. 2017 Effect of dietary essential oils of clove basil and ginger on Nile tilapia (*Oreochromis niloticus*) following challenge with *Streptococcus agalactiae*. *Aquaculture*, *468*: 235-243. **http://dx.doi.org/10.1016/j.aquaculture.2016.10.020**.

CHAGAS, E.C.; MACIEL, P.; AQUINO-PEREIRA, S.L. 2015 Infecções por acantocéfalos: um problema para a produção de peixes. In: DIAS, M.T.; MARIANO, W.S. *Aquicultura no Brasil: novas perspectivas*. São Carlos: Pedro & João Editores. v. 1, p. 305–328.

CHAKRABORTY, S.B.; HANCZ, C. 2011 Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. *Reviews in Aquaculture*, *3*(3): 103-119. **http://dx.doi.org/10.1111/j.1753-5131.2011.01048.x**.

DAS, R.; RAMAN, R.P.; SAHA, H.; SINGH, R. 2015 Effect of *Ocimum sanctum* Linn. (Tulsi) extract on the immunity and survival of *Labeo rohita* (Hamilton) infected with *Aeromonas hydrophila*. *Aquaculture Research*, *46*(5): 1111-1121. **http://dx.doi.org/10.1111/are.12264**.

FIGUEIREDO, H.C.P.; LEAL, C.A.G. 2008 Tecnologias aplicadas em sanidade de peixes. *Revista Brasileira de Zootecnia, 37*: 8-14. Suplemento Especial. http://dx.doi.org/10.1590/S1516-35982008001300002.

HAMILTON, M.A.; RUSSO, R.C.; THURSTON, R.V. 1977 Trimed Spearman–Karber method for estimating medial lethal concentrations in toxicity bioassays. *Environmental Science & Technology*, *11*(7): 714-719. **http://dx.doi.org/10.1021/es60130a004**.

HASHIMOTO, G.S.O.; MARINHO NETO, F.; RUIZ, M.L.; ACCHILE, M.; CHAGAS, E.C.; CHAVES, F.C.M.; MARTINS, M.L. 2016 Essential oils *of Lippia sidoides* and *Mentha piperita* against monogenean parasites and their influence on the hematology of Nile tilapia. *Aquaculture*, *450*: 182-186. **http://dx.doi.org/10.1016/j.aquaculture.2015.07.029**.

HELANDER, I.M.; NURMIAHO-LASSILA, E.L.; AHVENAINEN, R.; RHOADES, J.; ROLLER, S. 2001 Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. *International Journal of Food Microbiology*, *71*(2-3): 235-244. **http://dx.doi.org/10.1016/S0168-1605(01)00609-2**. PMid:11789941.

HENAO, J.; MUÑOZ, L.J.; PADILHA, L.; RÍOZ, E. 2010 Extraction and characterization of the essential oil of H.B.K. "Orégano de *Lippia origanoides* monte" cultivated at Quindío and evaluation of antimicrobial activity. *Research Journal University of Quindio*, *21*: 82-86.

IBGE – INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA 2016 *Produção da Pecuária Municipal*. Rio de Janeiro: IBGE. v. 44, 53 p.

IZEL, A.C.U.; CRESCÊNCIO, R.; O'SULLIVAN, F.F.L.A.; CHAGAS, E.C.; BOIJINK, C.L.; SILVA, J.I. 2013 *Produção intensiva de tambaqui em tanques escavados com aeração*. Manaus: Embrapa Amazônia Ocidental. 4 p. Circular Técnica, 39.

KUMAR, A.; RAMAN, R.P.; KUMAR, K.; PANDEY, P.K.; KUMAR, N.; MOHANTY, S.; KUMAR, A. 2012 *In vitro* and *in vivo* antiparasitic activity of Azadirachtin against *Argulus* spp. in *Carassius auratus* (Linn. 1758). *Parasitology Research*, *110*(5): 1795-1800. **http://dx.doi.org/10.1007/s00436-011-2701-0**. PMid:22042504.

LORENZI, H.; MATOS, F.J.A. 2008 *Plantas medicinais no Brasil: nativas e exóticas*. 2ª ed. Nova Odessa: Editora Instituto Plantarum. 544 p.

MAJOLO, C.; DA ROCHA, S.I.B.; CHAGAS, E.C.; CHAVES, F.C.M.; BIZZO, H.R. 2017 Chemical composition of *Lippia* spp. essential oil and antimicrobial activity against *Aeromonas hydrophila*. *Aquaculture Research*, *48*(5): 2380-2387. **http://dx.doi.org/10.1111/are.13073**.

MALHEIROS, D.F.; MACIEL, P.O.; VIDEIRA, M.N.; TAVARES-DIAS, M. 2016 Toxicity of the essential oil of *Mentha piperita* in *Arapaima gigas* (pirarucu) and antiparasitic effects on *Dawestrema* spp. (monogenea). *Aquaculture*, *455*: 81-86. **http://dx.doi.org/10.1016/j.aquaculture.2016.01.018**.

MARCIAL, G.; DE LAMPASONA, M.P.; VEGA, M.I.; LIZARRAGA, E.; VITURRO, C.I.; SLANIS, A.; JUÁREZ, M.A.; ELECHOSA, M.A.; CATALÁN, C.A.N. 2016 Intraspecific variation in essential oil composition of the medicinal plant *Lippia integrifolia* (Verbenaceae). Evidence for five chemotypes. *Phytochemistry*, *122*: 203-212. **http://dx.doi.org/10.1016/j.phytochem.2015.11.004**. PMid:26608668.

MEEPAGALA, K.M.; SCHRADER, K.K.; BURANDT, C.L. 2013 Antibacterial compounds from Rutaceae with activities against *Flavobacterium columnare* and *Streptococcus iniae*. *Journal of Agricultural Chemistry and Environment*, *2*(4): 90-100. **http://dx.doi.org/10.4236/jacen.2013.24014**.

MONTEIRO, P.C. 2012 *O uso do extrato aquoso de mastruz (Chenopodium ambrosioides L.) no controle de monogenóideos (Plathyhelminthes) em juvenis de tambaqui Colossoma macropomum (Cuvier, 1818), Manaus, Brasil*. Manaus. 76f. (Dissertação de Mestrado. Universidade Nilton Lins/INPA). Available from: <http://pgaquicultura.inpa.gov.br/pgaquicultura/images/Patrcia%20Castro.pdf> Access on: 7 oct. 2017.

OLIVEIRA, D.R.; LEITÃO, G.G.; BIZZO, H.R.; LOPES, D.; ALVIANO, D.S.; ALVIANO, C.S.; LEITÃO, S.G. 2007 Chemical and antimicrobial analyses of essential oil of *Lippia origanoides* H.B.K. *Food Chemistry*, *101*(1): 236-240. **http://dx.doi.org/10.1016/j.foodchem.2006.01.022**.

PILARSKI, F.; ROSSINI, A.J.; CECCARELLI, P.S. 2008 Isolation and characterization of *Flavobacterium columnare* from four tropical fish species in Brazil. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, *68*(2): 409-414. **http://dx.doi.org/10.1590/S1519-69842008000200025**. PMid:18660972.

POTZERNHEIM, M.C.L.; BIZZO, H.R.; SILVA, J.P.; VIEIRA, R.F. 2012 Chemical characterization of essential oil constituents of four populations of *Piper aduncum* L. from Distrito Federal, Brazil. *Biochemical Systematics and Ecology*, *42*: 25-31. **http://dx.doi.org/10.1016/j.bse.2011.12.025**.

RATTANACHAIKUNSOPON, P.; PHUMKHACHORN, P. 2010a Use of Asiatic pennywort *Centella asiatica* aqueous extract as a bath treatment to control columnaris in Nile tilapia. *Journal of Aquatic Animal Health*, *22*(1): 14-20. **http://dx.doi.org/10.1577/H09-021.1**. PMid:20575361.

RATTANACHAIKUNSOPON, P.; PHUMKHACHORN, P. 2010b Effect of *Cratoxylum formosum* on innate immune response and disease resistance against *Streptococcus agalactiae* in tilapia *Oreochromis niloticus*. *Fisheries Science*, *76*(4): 653-659. **http://dx.doi.org/10.1007/s12562-010-0242-6**.

REVERTER, M.; BONTEMPS, N.; LECCHINI, D.; BANAIGS, B.; SASAL, P. 2014 Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. *Aquaculture*, *433*: 50-61. **http://dx.doi.org/10.1016/j.aquaculture.2014.05.048**.

SANTOS, F.J.B.; LOPES, J.A.D.; CITO, A.M.G.L.; OLIVEIRA, E.H.; DE LIMA, S.G.; REIS, F.A.M. 2004 Composition and Biological Activity of Essential Oils from *Lippia origanoides* HBK. *The Journal of Essential Oil Research*, *16*(5): 504-506. **http://dx.doi.org/10.1080/10412905.2004.9698782**.

SEBASTIÃO, F.A.; FURLAN, L.R.; HASHIMOTO, D.T.; PILARSKI, F. 2015 Identification of bacterial fish pathogens in Brazil by direct colony PCR and 16S rRNA gene sequencing. *Advances in Microbiology*, *5*(6): 409-424. **http://dx.doi.org/10.4236/aim.2015.56042**.

SENA, A.C.; TEIXEIRA, R.R.; FERREIRA, E.L.; HEINZMANN, B.M.; BALDISSEROTTO, B.; CARON, B.O.; SCHMIDT, D.; COUTO, R.C.; COPATTI, C.E. 2016 Essential oil from *Lippia alba* has anaesthetic activity and is effective in reducing handling and transport stress in tambacu (*Piaractus mesopotamicus* x *Colossoma macropomum*). *Aquaculture*, *465*: 374-379. **http://dx.doi.org/10.1016/j.aquaculture.2016.09.033**.

SILVA, L.L.; BALCONI, L.S.; GRESSLER, L.T.; GARLET, Q.I.; SUTILI, F.J.; VARGAS, A.P.C.; BALDISSEROTTO, B.; MOREL, A.F.; HEINZMANN, B.M. 2017 S-(+)- and R-(-)-linalool: a comparison of the *in vitro* anti-*Aeromonas hydrophila* activity and anesthetic properties in fish. *Anais da Academia Brasileira de Ciências*, *89*(1): 203-212. **http://dx.doi.org/10.1590/0001-3765201720150643**. PMid:28423080.

SOARES, B.V.; CARDOSO, A.C.F.; CAMPOS, R.R.; GONCALVES, B.B.; SANTOS, G.G.; CHAVES, F.C.M.; CHAGAS, E.C.; TAVARES-DIAS, M. 2017a Antiparasitic, physiological and histological effects of the essential oil of *Lippia origanoides* (Verbenaceae) in native freshwater fish *Colossoma macropomum*. *Aquaculture (Amsterdam, Netherlands)*, *469*: 72-78. **http://dx.doi.org/10.1016/j.aquaculture.2016.12.001**.

SOARES, B.V.; NEVES, L.R.; FERREIRA, D.O.; OLIVEIRA, M.S.B.; CHAVES, F.C.M.; CHAGAS, E.C.; GONÇALVES, R.A.; TAVARES-DIAS, M. 2017b Antiparasitic activity, histopathology and physiology of *Colossoma macropomum* (tambaqui) exposed to the essential oil of *Lippia sidoides* (Verbenaceae). *Veterinary Parasitology*, *234*: 49-56. **http://dx.doi.org/10.1016/j.vetpar.2016.12.012**. PMid:28115182.

SOARES, B.V.; NEVES, L.R.; OLIVEIRA, M.S.B.; CHAVES, F.C.M.; DIAS, M.K.R.; CHAGAS, E.C.; TAVARES-DIAS, M. 2016 Antiparasitic activity of the essential oil of *Lippia alba* on ectoparasites of *Colossoma macropomum* (tambaqui) and its physiological and histopathological effects. *Aquaculture*, *452*: 107-114. **http://dx.doi.org/10.1016/j.aquaculture.2015.10.029**.

SOUZA, C.F.; BALDISSERA, M.D.; SANTOS, R.C.V.; RAFFIN, R.P.; BALDISSEROTTO, B. 2017b Nanotechnology Improves the therapeutic efficacy of *Melaleuca alternifolia* essential oil in experimentally infected *Rhamdia quelen* with *Pseudomonas aeruginosa*. *Aquaculture*, *473*: 169-171. **http://dx.doi.org/10.1016/j.aquaculture.2017.02.014**.

SOUZA, R.C.; COSTA, M.M.; BALDISSEROTTO, B.; HEINZMANN, B.M.; SCHMIDT, D.; CARON, B.O.; COPATTI, C.E. 2017a Antimicrobial and synergistic activity of essential oils of *Aloysia triphylla* and *Lippia alba* against *Aeromonas* spp. *Microbial Pathogenesis*, *113*: 29-33. **http://dx.doi.org/10.1016/j.micpath.2017.10.013**. PMid:29038058.

SUTILI, F.J.; CUNHA, M.A.; ZIECH, R.E.; KREWER, C.C.; ZEPPENFELD, C.C.; HELDWEIN, C.G.; GRESSLER, L.T.; HEINZMANN, B.M.; VARGAS, A.C.; BALDISSEROTTO, B. 2015a *Lippia alba* essential oil promotes survival of silver catfish (*Rhamdia quelen*) infected with *Aeromonas* sp. *Anais da Academia Brasileira de Ciências*, *87*(1): 95-100. **http://dx.doi.org/10.1590/0001-3765201520130442**. PMid:25789790.

SUTILI, F.J.; KREUTZ, L.C.; NORO, M.; GRESSLER, L.T.; HEINZMANN, B.M.; VARGAS, A.C.; BALDISSEROTTO, B. 2014 The use of eugenol against *Aeromonas hydrophila* and its effect on hematological parameters in silver catfish (*Rhamdia quelen*). *Veterinary Immunology and Immunopathology*, *157*(3-4): 142-148. **http://dx.doi.org/10.1016/j.vetimm.2013.11.009**. PMid:24368084.

SUTILI, F.J.; SILVA, L.L.; GRESSLER, L.T.; GRESSLER, L.T.; BATTISTI, E.K.; HEINZMANN, B.M.; VARGAS, A.C.; BALDISSEROTTO, B. 2015b Plant essential oils against *Aeromonas hydrophila*: *in* vitro activity and their use in experimentally infected fish. *Journal of Applied Microbiology*, *119*(1): 47-54.

TAVARES-DIAS, M. 2015 Parâmetros sanguíneos de referência para espécies de peixes cultivados. In: TAVARES-DIAS, M.; MARIANO, W.S. *Aquicultura no Brasil: novas perspectivas*. São Carlos: Editora Pedro & João. p. 11–30.

TAVARES-DIAS, M.; MARTINS, M.L. 2017 An overall estimation of losses caused by diseases in the Brazilian fish farms. *Journal of Parasitic Diseases : Official Organ of the Indian Society for Parasitology*, *41*(4): 913-918. **http://dx.doi.org/10.1007/s12639-017-0938-y**. PMid:29114119.

THOMAS, J.; JEROBIN, J.; SEELAN, T.S.J.; THANIGAIVEL, S.; VIJAYAKUMAR, S.; MUKHERJEE, A.; CHANDRASEKARAN, N. 2013 Studies on pathogenicity of *Aeromonas salmonicida* in catfish *Clarias batrachus* and control measures by neem nanoemulsion. *Aquaculture*, *396-399*: 71-75. **http://dx.doi.org/10.1016/j.aquaculture.2013.02.024**.

VALLADÃO, G.M.R.; GALLANI, S.U.; PILARSKI, F. 2016 South American fish for continental aquaculture. *Reviews in Aquaculture*, 1-19. http://dx.doi.org/**10.1111/raq.12164**.

VICUNA, G.C.; STASHENKO, E.E.; FUENTES, J.L. 2010 Chemical composition of the *Lippia origanoides* essential oils and their antigenotoxicity against bleomycin-induced DNA damage. *Fitoterapia*, *81*(5): 343-349. **http://dx.doi.org/10.1016/j.fitote.2009.10.008**. PMid:19874875.

YAO, J.; LI, X.; SHEN, J.; PAN, X.; HAO, G.; XU, Y.; YING, W.; RU, H.; LIU, X. 2011 Isolation of bioactive components from *Chelidonium majus* L. with activity against *Trichodina* sp. *Aquaculture*, *318*(1-2): 235-238. **http://dx.doi.org/10.1016/j.aquaculture.2011.04.035**.

ZHENG, Z.L.; TAN, J.Y.W.; LIU, H.Y.; ZHOU, X.H.; XIANG, X.; WANG, K.Y. 2009 Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growth, antioxidant effect and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*). *Aquaculture*, *292*(3-4): 214-218. **http://dx.doi.org/10.1016/j.aquaculture.2009.04.025**.