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BIOLOGY

Essential oil of *Lippia alba* in the transport of Nile tilapia

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ABSTRACT: *This study aimed to examine the action of the essential oil of **Lippia alba** (EOLA) in the stress response for transport of Nile tilapia **Oreochromis niloticus**. The fish were transported into three treatments (in triplicate): control, 10 and 20 µL L^{-1} EOLA, with loading density of 15 fish/plastic bags for 8h. Plasma glucose levels were significantly decreased in fish exposed to 20µL L^{-1} EOLA in comparison with the control group and fish exposed to 10µL L^{-1} EOLA, but the plasma cortisol, lactate and paraoxonase levels were similar. Un-ionized ammonia and ventilatory rate demonstrated a significant reduction in the treatments with the use of EOLA. In conclusion the use of 20µL L^{-1} EOLA is indicated for Nile tilapia transport.*
Key words: ***Oreochromis niloticus**, stress, glucose, cortisol, ventilatory rate.*

Óleo essencial de *Lippia alba* no transporte de tilápia-do-Nilo

RESUMO: *O estudo objetivou verificar a ação do óleo essencial de **Lippia alba** (EOLA) na resposta de estresse para o transporte de tilápia-do-Nilo **Oreochromis niloticus**. Os peixes foram transportados por 8h sob três tratamentos (triplicata): controle, 10 e 20µL L^{-1} EOLA. Níveis plasmáticos de glicose foram significativamente menores em peixes expostos a 20µL L^{-1} de EOLA, em relação com o grupo controle e os peixes expostos a 10µL L^{-1} de EOLA, mas, cortisol, lactato e paraoxonase plasmáticos foram similares. Amônia não-ionizada e taxa de ventilação demonstraram redução significativa nos tratamentos com EOLA. Conclui-se que o uso de 20µL L^{-1} de EOLA é indicado para o transporte de tilápia-do-Nilo.*
Palavras-chave: ***Oreochromis niloticus**, estresse, glicose, cortisol, taxa de ventilação.*

In aquaculture, the transportation of live fish is routinely performed. Addition of essential oil of ***Lippia alba*** (EOLA) to the water during transport has the positive effect of stress reduction in silver catfish ***Rhamdia quelen*** Quoy & Gaimard, 1824 (BECKER et al., 2012) and tambacu (***Piaractus mesopotamicus*** × ***Colossoma macropomum***) (SENA et al., 2016).

Lippia alba is a medicinal plant native to South America; EOLA is safe for consumers and the environment and has been demonstrated to have sedative effect (HOHLENWERGER et al., 2016). There are no studies reporting the effects of EOLA on the Nile tilapia ***Oreochromis niloticus*** transport and the goal of this study was to verify the efficacy of EOLA in the reduction of stress in the transport of Nile tilapia.

The EOLA was obtained from the fresh leaves of ***L. alba*** by hydrodistillation and the main chemical constituents were linalool (47.66%), β-myrcene (11.02%) and eucalyptol (9.77%) (HOHLENWERGER et al., 2016). Juveniles of Nile tilapia (80.79±6.69g and 16.69±1.43cm) were purchased from Bahia Pesca (Camaçari, Brazil) and were transported by car for 8h in nine plastic bags with 8L of water and 8L of oxygen (15 fish per plastic bag), and they were divided into three treatments (three replicates each): 0 (control); 10 and 20µL L^{-1} of EOLA (both first diluted in ethanol 1:10). Another 15 fish were not subjected to transport (designed "before transport") and remained in water free of EOLA throughout the trial. The concentrations of EOLA

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were within the range suggested for the transport of Nile tilapia (HOHLENWERGER et al., 2016). Water parameters of temperature (ºC), dissolved oxygen (mg L^{-1} O_2), pH, hardness (mg L^{-1} $CaCO_3$), alkalinity (mg L^{-1} $CaCO_3$), nitrite (N-NO_2) and un-ionized ammonia (N-NH_3) were determined before and after transportation.

Blood samples were collected from the caudal vein before and 8h after transport (each fish was sampled once) using a heparinized syringe, transferred to 2mL plastic tubes and centrifuged at 3000xg (6ºC, 15min) to separate the plasma. Cortisol S kit was used for the determination of cortisol in the plasma aliquots in the mini-VIDAS® equipment. The plasma glucose levels were determined enzymatically by glucose oxidase (GOD)/glucose peroxidase (POD) in BT 3000 Plus (500 tests $hour^{-1}$; Wiener Lab, Rosario, Argentina). The plasma lactate levels were determined using a fully automatic analyzer. The paraoxonase activity was carried out by measuring p-nitrophenol in spectofotometer.

To test the possible effect by inhibition of the respiratory system of EOLA, eight fish (an individual for 10L aquarium) that were not submitted to transport were used per treatment under the same EOLA concentrations of transport treatments to evaluate the ventilation rate (VR) in 0; 0,5; 1; 2; 3, 4, 5, 6, 7 and 8h of exposure and each juvenile was used only once. The VR was quantified by visually counting 20 successive opercular/buccal movements, measuring the elapsed time with a chronometer (adapted from BECKER et al., 2012).

All data are expressed as the mean ± SEM and were subjected to a Levene test. Because the data exhibited homogeneous variances, comparisons between different treatments and times were made using a one-way ANOVA followed by Tukey tests. Significance was set at a critical level of 95% ($P<0.05$), with a confidence interval of 95%.

No mortality in the fish was observed in this study. After transport, un-ionized ammonia in the control group (1.33±0.17µg L^{-1} N-NH_3) was significantly higher than in other treatments (0.39±0.04 and 0.45±0.06µg L^{-1} N-NH_3 for 10 and 20µL L^{-1} EOLA, respectively) ($P<0.05$). Other water parameters were not affected significantly by treatments: temperature (26.6±0.11, 26.2±0.15 and 26.7±0.15ºC), dissolved oxygen (7.46±0.47, 7.04±0.78 and 6.91±0.89mg L^{-1} O_2), pH (6.39±0.12, 5.81±0.14 and 5.93±0.09), hardness (50±0.01, 45±0.01 and 47.8±1.67mg L^{-1} $CaCO_3$), alkalinity (60.0±0.01, 70.0±0.01 and 61.67±2.88mg L^{-1} $CaCO_3$) or nitrite (0µg L^{-1} N-NO_2) for 0, 10 and 20µL L^{-1} EOLA, respectively.

Fish of the control group and transported with 10µL L^{-1} EOLA had significantly higher plasma glucose levels than fish transported with 20µL L^{-1} EOLA ($P<0.05$) (Figure 1A). The plasma cortisol (Figure 1B) and paraoxonase (Figure 1C) levels were similar in all groups at all times ($P<0.05$). The plasma lactate levels were lower than 0.5mmol L^{-1} for all fish and also did not show significant differences between treatments ($P<0.05$).

Fish exposed with EOLA (10 and 20µL L^{-1}) presented lower VR than the control group ($P<0.05$). Effect of EOLA on the VR was concentration-dependent at 0.5h and after 2h, with higher concentrations of EOLA associated with lower VRs (Table 1).

The EOLA used in this study was the same used by HOHLENWERGER et al. (2016), where linalool was the major component as in previous studies (BECKER et al., 2012; HELDWEIN et al., 2012); although, there were discrepancies between the compounds that presented minor proportions. The activity of the main component is influenced by other minor molecules (TEIXEIRA et al., 2016), suggesting that although there were differences between components, EOLA used in this study was as effective as EOLA used by BECKER et al. (2012) and HELDWEIN et al. (2012).

The use of EOLA may be useful in the transport of fish for long periods, as in this study. Additionally, the un-ionized ammonia levels observed in the present study were well below the tolerance limits for Nile tilapia. Besides, the water quality parameters measured in this study showed that there were no adverse effects to the exemplars after transport.

Fish have in response to stress rapid release of catecholamines (primary neuroendocrine response). Because of high levels of these hormones in the circulatory system, a wide range of secondary responses can be observed through disturbance of the metabolic parameters, like plasma glucose and cortisol (WENDELAAR-BONGA, 1997).

Hyperglycemia occurred in fish of the control group and transported with 10µL L^{-1} EOLA when compared to fish not submitted to transport. Possibly this increase in plasma glucose levels due to catecholamine mediated glycogenolysis may represent an adaptive response to stress caused by transport. Additionally, the concentration of plasma lactate levels indicated that the use of EOLA did not require the use of an anaerobic metabolic pathway as an important energy source through transport (PANKHURST, 2011).

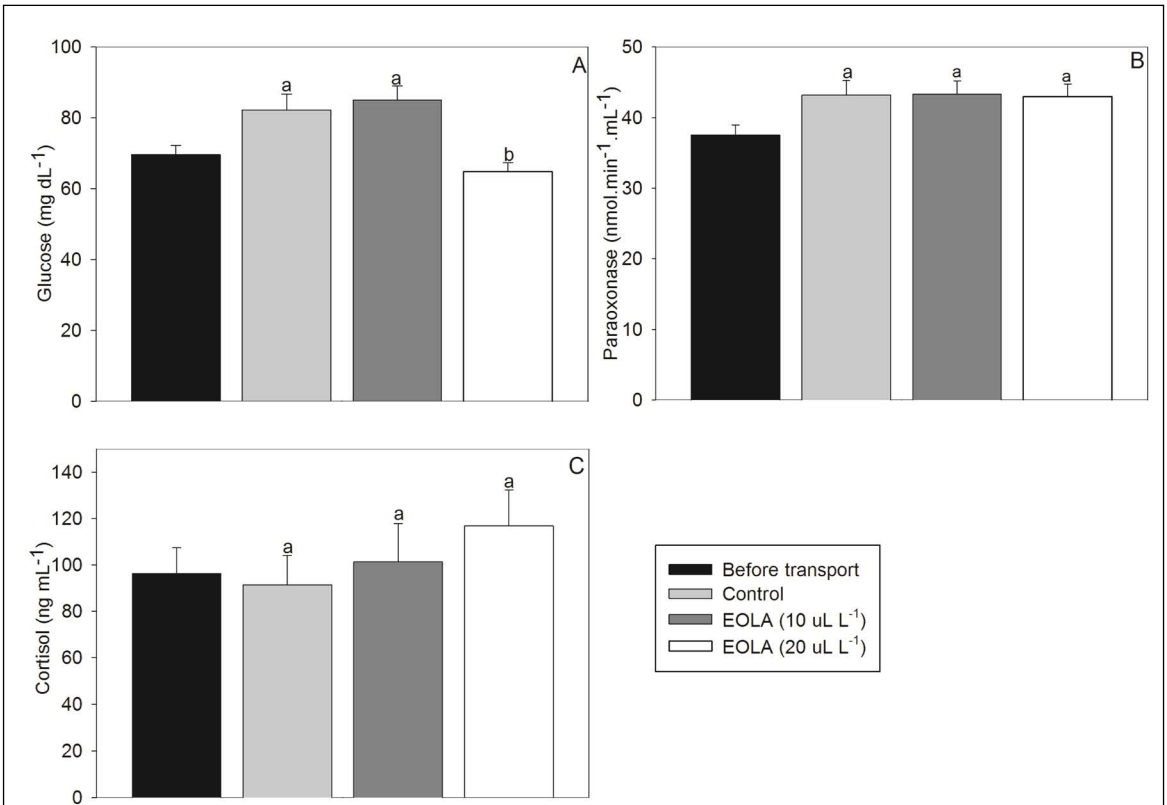

Figure 1 - Effect of the essential oil of ***Lippia alba*** (EOLA) added to the water on plasma glucose (A), paraoxonase (B) and cortisol (C) levels in Nile tilapia after 8 h transport. Different letters indicated significant difference between treatments (P<0.05).

Plasma paraoxonase is an important indicator of protection against oxidative stress, damage to the immune system and changes in total plasma proteins (MING et al., 2012). Nile tilapia can adapt to chronic stress situations, but the chronic stress response can provoke a reduction in performance and growth rates compared with non-stressed fish (BARCELLOS et al., 1999). In this study, plasma paraoxonase and cortisol levels indicated that exposure to EOLA does not alter the protection mechanism for stress.

VR in Nile tilapia transported with EOLA was lower than in control group, indicating a more effective prevention of the elevation of respiratory rate, which could contribute to a lower consumption of oxygen and stress. However, as plasma cortisol levels were not affected by the addition of EOLA in the water of transport, probably this oil reduced catecholamine release and/or metabolism, which led to the lower VR observed. Treatment 20µL L^{-1} EOLA showed VR decreasing over time with a minimum peak between 4 and 5h. So, 20µL L^{-1} of EOLA demonstrated more effective bradypnoea between 4-5h, can continue for at least 8h. Lower VR could result in sustained hypoxia, which was not observed in this study, since lactate levels remained stable. Similar to our results, silver

Table 1 - Effect of the essential oil of ***Lippia alba*** (EOLA) added to the water on ventilation rate in Nile tilapia (n=8). Different capital letters indicated significant differences between treatments at the same time. Different lowercase letters indicate significant differences between different times at the same treatment (P<0.05).

Time	----------Treatments----------		
	Control	EOLA 10µL L^{-1}	EOLA 20µL L^{-1}
0	146 ± 20.15^{Aa}	131 ± 19.42^{Aa}	129 ± 17.19^{Aa}
0.5	147 ± 19.80^{Aa}	97 ± 13.54^{Bb}	74 ± 9.49^{Cb}
1	135 ± 18.45^{Aa}	84 ± 11.31^{Bbc}	62 ± 7.87^{Bbc}
2	139 ± 20.02^{Aa}	79 ± 10.70^{Bbc}	47 ± 5.61^{Cc}
3	136 ± 19.62^{Aa}	71 ± 9.09^{Bc}	46 ± 5.76^{Cc}
4	130 ± 18.84^{Aa}	67 ± 8.63^{Bc}	41 ± 5.02^{Cd}
5	124 ± 18.52^{Aa}	67 ± 8.49^{Bc}	43 ± 5.19^{Cd}
6	135 ± 20.11^{Aa}	88 ± 13.35^{Bbc}	46 ± 5.77^{Cc}
7	140 ± 19.77^{Aa}	75 ± 9.52^{Bbc}	50 ± 6.53^{Cc}
8	143 ± 21.08^{Aa}	74 ± 9.64^{Bbc}	49 ± 5.94^{Cc}

catfish with addition of EOLA (10 or 20μL L^{-1}) also decreased VR (BECKER et al., 2012).

In conclusion, the use of 20μL L^{-1} of EOLA is safe and suitable for Nile tilapia transport, primarily due to reduce plasma glucose, VR and un-ionized ammonia.

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