RBSPA
Revista Brasileira de Saúde e Produção Animal

ISSN 1519-9940
Revista Brasileira de Saúde e Produção Animal
http://doi.org/10.1590/S1519-994020240005

Received: 10/02/2024
Accepted: 14/06/2024

Fishing Resources / Aquaculture
Editor: José Givanildo da Silva

Aloe vera as a behavior modulator for *Moenkhausia forestti* submitted to transport

Aloe vera como modulador de comportamento em Moenkhausia forestti submetido ao transporte

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ABSTRACT The ornamental aquaculture market has been expanding, and the transport of living organisms in aquaculture is constant, which affects the behavior and health of these organisms. Due to a lack of methods, small fish species are little studied in ornamental aquaculture. Therefore, the use of bioactives can promote positive responses for the management of these species. This study evaluated the behavior of *Moenkhausia forestti* transported with Aloe vera powder and Aloe vera gel added to the water. A previous assessment of O_2 standards in water with the inclusion of Aloe vera was carried out by measuring dissolved oxygen and pH. An increase in dissolved oxygen was observed over time in treatments with added Aloe vera gel, while a drop in oxygen was observed in treatments with Aloe vera powder after 60 minutes. The fish were subjected to the Control, Aloe vera gel, and Aloe vera powder treatments with 10 fish/bag per repetition. They were transported for 4 hours, and behaviors were evaluated immediately after the transport and after 12 and 24 hours upon arrival. The water conditions and transportation did not significantly affect mortality. The use of Aloe vera powder or Aloe vera gel during transport led to a reduction in stress-related behaviors such as erratic swimming, chasing, and biting.

Keywords aquaculture, bioactive, plant, stress, water quality

RESUMO *O mercado de aquicultura ornamental vem se expandindo e o transporte de organismos vivos na aquicultura é constante, o que afeta o comportamento e a saúde desses organismos. Devido à falta de métodos, espécies de peixes de pequeno porte são pouco estudadas na aquicultura ornamental. Portanto, o uso de bioativos pode promover respostas positivas para o manejo dessas espécies. Este estudo avaliou o comportamento de Moenkhausia forestti transportada com pó de Aloe vera e gel de Aloe vera adicionado à água. Uma avaliação prévia dos padrões de O_2 em água com a inclusão de Aloe vera foi realizada medindo-se oxigênio dissolvido e pH. Um aumento no oxigênio dissolvido foi observado ao longo do tempo nos tratamentos com adição de gel de Aloe vera, enquanto uma queda no oxigênio foi observada nos tratamentos com pó de Aloe vera após 60 minutos. Os peixes foram submetidos aos tratamentos Controle, Gel de Aloe vera e Aloe vera em pó com 10 peixes/saco por repetição. Eles foram transportados por 4 horas e os comportamentos foram avaliados imediatamente após o transporte e após 12 e 24 horas na chegada. As condições hídricas e de transporte não afetaram significativamente a mortalidade. O uso de pó de Aloe vera ou gel de Aloe vera durante o transporte levou a uma redução nos comportamentos relacionados ao estresse, como natação errática, perseguição e mordida.*

Palavras-chave *aquicultura, bioativo, plantas, estresse, qualidade da água*

1. Introduction

In aquaculture, the transportation of living organisms is a constant activity (Fantini et al., 2020) that affects the behavior and health of aquatic organisms (Goes et al., 2018). The ornamental aquaculture market has been expanding steadily over the years, showing an estimated amount of 2 billion fish transported/year (Monticini, 2010). However, small species are little studied in ornamental aquaculture due to a lack of methods because of the difficulties in managing these fish; they are as sensitive as larvae, and the challenge of obtaining biological material to carry out analyses.

Studies that describe the action of stress-mitigating substances use tools for measuring glucose and plasma cortisol as indicators (Acerete et al., 2004; Honorato et al., 2014; Goes et al., 2018). The opportunity to use other techniques, such as behavior change (Berlinghieri et al., 2021), is relevant because it allows applicability in the production sector, commerce, breeders, and hobbyists (Vanderzwalmen et al., 2019).

The study of animal behavior can be a crucial tool in aquaculture (Laursen et al., 2011). Small fish species are little studied due to the difficulty in collecting biological material (Deboleto et al., 2020) for analyses commonly used to detect well-being and stress (Fernández-Alacid et al., 2019). The use of bioactives can promote positive responses in management (Ota et al., 2019); however, the responses are related to the species, type, and duration of the stress (Ferreira et al., 2021).

Among the plants that can be used in transport is Aloe vera (*Aloe barbadensis*), which has anti-inflammatory, antioxidant, and immunostimulating characteristics in addition to fungicidal activity (Pereira et al., 2016). It has been used to transport Matrinxã (*Brycon amazonicus*) (Zanuzzo et al., 2017) with positive results that can be tested on other fish species. *Moenkhausia forestti*, a freshwater tetra popularly known as the fire-eye tetra, is among the species from the Brazilian Pantanal involved in a few studies on its management with a focus on the aquarium hobby.

This study evaluated the effects of Aloe vera powder and Aloe vera gel when added to the transportation water, on the behavior of *Moenkhausia forestti* to identify fish behaviors that demonstrate exposure to stress.

2. Material and methods

The study was carried out after approval by the Animal Use Ethics Committee (CEUA/UFGD) of the Federal University of Grande Dourados, located in Dourados - MS, under protocol 34/2017 of 11/17/2019, which involves the production, maintenance, and/or use of animals belonging to the phylum *Chordata*, subphylum *Vertebrata* (except humans), for scientific research purposes, and specifically using the native species *Moenkhausia forestti*, popularly known as fire-eye tetra.

2.1. Obtaining extracts and characterization of the products

Aloe vera leaves were collected in Dourados - MS, at the geographic coordinates of Latitude 22° 13' 18" South and Longitude 54° 48' 23" West. The leaves were selected, washed, sanitized with drinking water, and suspended for 12 min to remove the lanolin. They were later peeled, pulped, and processed to obtain a homogeneous gel. The powdered extract was purchased commercially (Kampo de Ervas Indústria e Comércio Ltda-ME, Ribeirão Preto - SP, Brazil), in which Aloe vera is the only ingredient. The products were stored in a freezer until use.

2.2. Experiment 1: Measurement of dissolved O_2 in water added with Aloe vera

Different concentrations of Aloe vera-based products were used to measure the dissolved oxygen and pH as parameters that affect the water quality. Glass containers with a capacity of 2 L were used with a volume of 1 L of water in a static system with constant aeration. These remained in an environment with a controlled temperature (28 ºC) and were exposed to light during the test.

It has been performed 3 treatments: Control, Aloe vera gel, and Aloe vera powder. The Control treatment was carried out without the addition of product (water only), while in the Aloe vera gel treatment, the gel was used in concentrations of 2, 4, 6, and 8 ml per liter of water, in which the concentrations were based on the use of the commercial conditioner Labcon Protect Plus, which is applied during the transport of the fish and the Aloe vera powder treatment, it was used in concentrations of 0.2 g, 0.4 g, 0,6 g and 0.8 grams of powder per liter of water.

Measurements of dissolved O_2 in the water were taken at 0, 15, 30, 60, and 90 minutes after the start of transportation. Tests were performed in triplicates. The experiment was based on a

completely randomized design, using a split-plot scheme, consisting of three treatments (Control, Aloe vera gel, and Aloe vera powder).

2.3. Experiment 2: Behavior analysis of Moenkhausia forestti with Aloe vera powder and gel

Adult *Moenkhausia forestti* (mixed sex; 4.4 ± 0.82 cm and 1.43 ± 0.82 g) from existing stock in the IMASUL Ichthyology laboratory were kept in a 2000 L collective aquarium with environmental enrichment and a recirculation system with charcoal biological and UV filtration (temperature: 23.5 ± 0.02 °C; pH: 7.17 ± 0.03; dissolved oxygen: 7.47 ± 0.03 mg/L). The fish were transferred to a system composed of 20 L experimental units with a density of 10 fish for a period of 72 h to be transported.

After the acclimatization period, the fish were subjected to transport that lasted for 4 hours, which is a highly stressful factor. The fish were transported in groups of 10 in transparent polyethylene bags (50 x 70 cm) containing 3 L of water from the recirculation system. Three bags of each treatment category were composed as follows:

The treatments, with three replications each, were composed of the non-addition of the product (water only; Control), Aloe vera gel at a concentration of 8 mL/L (Aloe vera gel), and Aloe vera powder at a concentration of 0.2 g/L (Aloe vera powder).

The bags were sealed with air in the space at the top of the bag and placed in a closed bucket during transportation. After the stress of transport, the fish were transferred by net in their groups to the system they belonged to, and their behavior was analyzed immediately after the transport (PT), 12 (12R), and 24 hours (24R) after arrival.

The water pH, oxygen, and temperature were measured at each sampling time point. Dissolved oxygen and temperature were measured using a portable oxygen meter (Ysi 550 A - YSI incorporated®, Yellow Springs, OH, USA), pH was determined using a pH meter (HI8314 - Hanna Instruments, Barueri, SP, Brazil), ammonia was measured using a colorimetric kit (Alfakit®, Florianopolis, SC, Brazil), and conductivity was measured with the digital hydroponic Ppm & Tds sensor (Ppm – Part Per Million; Tds - Total Dissolved Solids; TDS & EC, Fujian, China).

2.3.1. Behavioral analysis

The behaviors of each fish were evaluated to obtain the characteristics. Any visible injury to the body and fin was recorded for focal fish and given a severity score from * to +++, where (*) means no visible injury or unusual behavior; (+) mild non-life-threatening injury or unusual behavior; (++) average non-life-threatening injury or unusual behavior; (+++) serious injury or unusual behavior with possible risk to life (Vanderzwalmen et al., 2020a). Scores for fins and body injuries were summed to obtain a total injury score recorded for each fish (Table 1).

2.4. Statistical analysis

A correspondence analysis (Manly, 2008) was initially performed to verify whether behavioral patterns are altered due to the presence of the extracts. The analysis combined the frequencies of behavioral units associated with basic posture and agonism between individuals in the control and experimental groups. Subsequently, these were subjected to analysis of variance (ANOVA). The Tukey's test was applied to evaluate the behavior scores. The ANOVA assumptions were examined using the Shapiro-Wilk test for data normality and Levene's test for homogeneity of variances.

3. Results

3.1. Experiment 1: O_2 patterns in the water with Aloe vera inclusion

No mortality in the *Moenkhausia forestti* exposed to the Aloe vera conditions was observed during the laboratory test. The water quality parameters with the presence of Aloe vera in gel showed

an increase in dissolved oxygen over time with pH at 7.85 ± 0.45 when adapting the data to a quadratic equation. The water with the presence of Aloe vera in powder showed a drop in oxygen and a pH of 7.6 ± 0.21 after 60 minutes of exposure (Figure 1).

Table 1 – List of recorded behaviors of *Moenkhausia forestti*.

Behavior	Presentation	Well-being description
Chasing	It occurs when fish are chasing each other	Aggressive interactions are related to high stress (Gronquist & Berges, 2013)
Biting	It occurs when fish are biting each other	Bites can cause injuries and death (Noble et al., 2012)
Erratic swimming	It occurs through rapid swimming and changing direction when the chasing stops	Irregular swimming is an indicator of high stress, distress, or pathogenic status and can be used as a signal for reduced health (Gronquist & Berges, 2013)

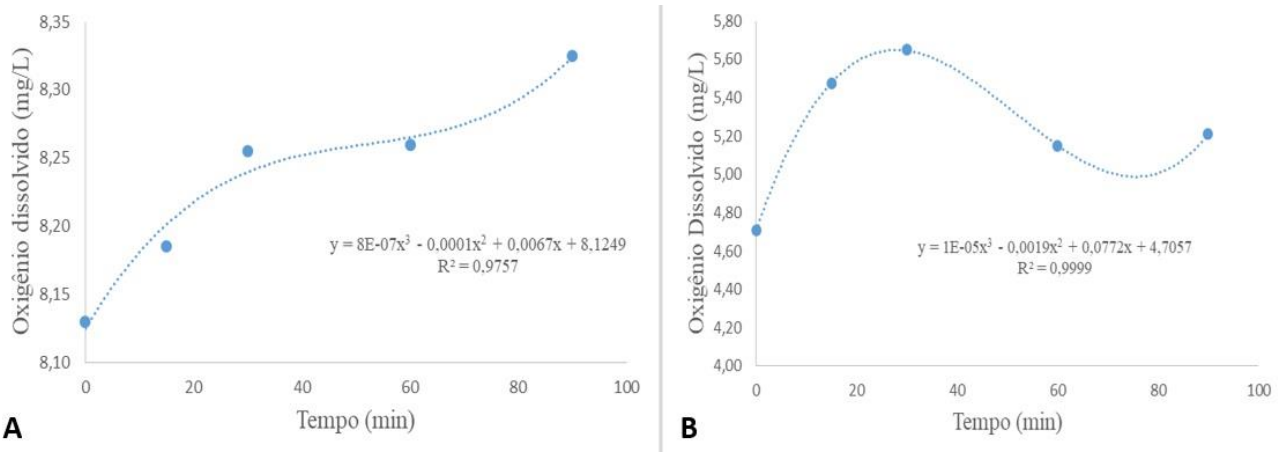

Figure 1 – O_2 dissolved patterns in the water with Aloe vera gel (A) or Aloe vera powder (B).

The viscosity of Aloe vera gel and powder in the presence of water was not effective to change the water quality characteristics that reflect the survival of fish.

3.2. Experiment 2: Behaviour analysis

The water parameters in the bags upon arrival at the laboratory were: pH: 7.0 ± 0.23; oxygen: 11.07 ± 4.67; temperature: 25 °C ± 1.45; conductivity 0.170 uS/cm ± 0.012; and ammonia 0.25 ppm ± 0.02 (mean ± SD). The water conditioner and transport stage did not significantly affect mortality, which was low throughout the experiment.

Fish that were handled without the use of Aloe vera additives showed behaviors with a high incidence of erratic swimming, chasing, and biting, which demonstrates stress behaviors during transport management. The use of Aloe vera powder or Aloe vera gel reduced these behaviors.

The use of Aloe vera gel seemed to have provided increased comfort to the fish because no stress behavior was observed at 12 and 24 hours after transport (Table 2).

Table 2 – Behavioral analyses of *M. forestti* fish before and after 4-hour transport, submitted to Control, Aloe vera powder, and Aloe vera gel treatments.

Treatments		Behavior[1]		
		Erratic swimming	Chasing	Biting
Control	Before transportation	*	*	*
	After transportation	++	++	++
	At 12h of recovery	+	+	+
	At 24h of recovery	*	*	*
Aloe vera powder	Before transportation	*	*	*
	After transportation	++	++	++
	At 12h of recovery	*	+	+
	At 24h of recovery	*	*	*
Aloe vera gel	Before transportation	*	*	*
	After transportation	*	+	+
	At 12 h of recovery	*	*	*
	At 24 h of recovery	*	*	*

[1] *: no clinical signs; +: mild clinical signs; ++: moderate clinical signs; +++: severe clinical signs.

After the transport, changes in pH and dissolved oxygen parameters were observed at 12 and 24 h time points, which are presented in Table 3. No significant changes were observed in temperature, ammonia, and conductivity.

Table 3 – Water analysis for pH and dissolved oxygen (mg/L) after 12 and 24 hours of transport of *M. forestti* fish, submitted to Control, Aloe vera powder, and Aloe vera gel treatments.

Parameter	Time	Treatments		
		Control	Aloe vera powder	Aloe vera gel
Dissolved oxygen	12 h	17.30 ± 1.41	17.70 ± 0.71	18.48 ± 0.11
	24 h	7.88 ± 0.07	7.84 ± 0.00	7.85 ± 0.16
pH	12 h	7.00 ± 0,00	7.00 ± 0,00	7.00 ± 0,00
	24 h	7.50± 0,00	7.50 ± 0,00	7.50 ± 0,00

4. Discussion

The quality of the water when transporting fish is a fundamental factor for the success of the operation (Pinheiro et al., 2021). Aloe vera promoted changes in water quality, with the use of Aloe vera gel being more pronounced (Table 3). It is noteworthy that gel formation changes the pH and viscosity characteristics of the diluent (Parente et al., 2013), which resulted in changes in the behavior of fish during transportation in this study.

Aloe vera has active substances in its composition, with antimicrobial, immunomodulatory, antitumor, hepatoprotective, nephroprotective, hypoglycemic, anti-inflammatory, antioxidant, gastroprotective, and wound healing (Mahdavi et al., 2013; Parente et al., 2013; Zanuzzo et al.,

2017). Gel formation is associated with the presence of polysaccharides, which can be beneficial for several functions but can impair gas exchange in fish (Syed et al., 2022; Zanuzzo et al., 2017).

Oxygen exchange functions may be affected by the inclusion of Aloe vera, with opercular heartbeat changes as indicative (Carraschi et al., 2011; Honorato et al, 2014). The change in the Opercular beat may be a consequence of the addition of substances in the water that promote irritation in the gills and increase the production of mucus, which leads to changes in gas exchange (Honorato et al., 2014).

Fish that were transported with Aloe vera added to the water showed a reduction in behaviors associated with stress, including erratic swimming and chasing. Erratic swimming in fish in the Aloe vera group was lower upon arrival after transportation and at rest compared to fish in the control group. Similarly, fish transported with Aloe vera showed lower levels of chasing during the recovery time.

Previous studies showed that the behavioral escape response visually mediated (Ren et al., 2002) is a stress response (Braithwaite and Ebbesson, 2014; Vanderzwalmen et al., 2020a). Some levels of chasing and biting are probably unavoidable when ornamental fish are placed in a new community (White et al., 2017) or new environments after transport (Gronquist & Berges, 2013).

Aggressive behavior characterized by chasing and biting among schools of fish can result in chronic stress (Vanderzwalmen et al., 2020b). Thus, reduced aggressive behavior can be an indication of good environmental conditions and fish welfare (White et al., 2017). Behaviors resulting from the establishment of hierarchy can increase the risk of skin injuries due to bites (Wells, 2009; Vanderzwalmen et al., 2020b; Jones et al., 2023).

The recovery time from stress can extend for weeks (Fernström et al., 2008); therefore, the use of environmental enrichment (Vanderzwalmen et al., 2020b) and herbal substances (Ventura et al., 2019; Zago et al., 2023; Bodur et al., 2024 is recommended and can be important in mitigating the deleterious effects of aggressive behavior.

4. Conclusions

The inclusion of Aloe vera gel in the transportation water is recommended for *Moenkhausia forestti* to mitigate the behavioral effects associated with stress.

Declaration of Conflict of Interest

The authors declared no conflicts of interest.

Authorship Contribution Statement

Claudia Aparecida Honorato: Supervision, Conceptualization, Writing – original draft, Project administration, Data curation; **Annye Campos Venâncio Ferreira:** Writing – review & editing, Data curation; **José Guilherme Camargo Coneglian:** Investigation, Visualization; **Mayara Schueroff Siqueira:** Visualization, Software; **Joyce Zanella:** Formal analysis, Investigation; **Jéssica Amanda Ugarte Reges:** Investigation, Visualization, Formal Analysis; **Heriberto Gimênes Júnior:** Conceptualization, Validation, Resources; **Weliton Vilhalba da Silva:** Methodology, Visualization.

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