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Short Communication

Therapeutic bath of mint hydrolate in the control of monogenea for four tilapia species

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ABSTRACT

This work aimed to evaluate a therapeutic bath of mint (*Mentha x villosa*) hydrolate in the control of monogeneans for four tilapia species (*Oreochromis niloticus*, *Oreochromis aureus*, *Oreochromis mossambicus*, and *Oreochromis hornorum*) reared in the same environment (pond). After two months, 60 individuals of *O. niloticus* were divided into six groups of ten fish each. Three of them were submitted to a 1-hour therapeutic bath of mint hydrolate at the concentration of 20 mL·L^{-1}, and the others were used as a control group. This process was repeated for *O. aureus*, *O. mossambicus* and *O. hornorum*. After that, mucus and gills were analyzed to determine parasitological indices (prevalence, mean abundance, and mean intensity) for monogeneans. The efficacy of mint hydrolate bath was set as 73.5, 79, 80.7, and 84.5% for *O. hornorum*, *O. aureus*, *O. mossambicus* and *O. niloticus*, respectively, against the monogeneans in the mucus. All species in this work demonstrated similar parasitic susceptibility when reared in the same environment. In addition, the use of mint therapeutic bath demonstrated efficacy in the control of monogeneans in mucus for all evaluated species.

Keywords: Fish health, Herbal medicines, *Mentha* x *Villosa*, Parasitology.

Banho terapêutico de hidrolato de hortelã no controle de parasitas para quatro espécies de tilápia

RESUMO

Este trabalho teve como objetivo avaliar um banho terapêutico de hidrolato de hortelã (*Mentha x villosa*) no controle de monogenéticos para quatro espécies de tilápia (*Oreochromis niloticus*, *Oreochromis aureus*, *Oreochromis mossambicus* e *Oreochromis hornorum*) criadas no mesmo ambiente (viveiro). Após dois meses, 60 indivíduos de *O. niloticus* foram divididos em seis grupos de dez peixes cada um. Três deles foram submetidos a um banho terapêutico de uma hora de hidrolato de hortelã na concentração de 20 mL·L^{-1}, e os demais foram utilizados como grupo controle. Esse processo foi repetido para *O. aureus*, *O. mossambicus* e *O. hornorum*. Em seguida, o muco e as brânquias foram analisados para determinar os índices parasitológicos (prevalência, abundância média e intensidade média). A eficácia do banho de hidrolato de hortelã no muco foi medida em 73,5, 79, 80,7 e 84,5% para *O. hornorum*, *O. aureus*, *O. mossambicus* e *O. niloticus*, respectivamente. Todas as espécies de tilápia avaliadas neste trabalho demonstraram suscetibilidade parasitária semelhante quando criadas no mesmo ambiente, no entanto o uso do banho terapêutico de hortelã demonstrou eficácia no controle de monogenéticos no muco para todas as espécies avaliadas.

Palavras-chave: Sanidade dos peixes, Fitoterápicos, *Mentha X Vilosa*, Parasitologia.

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INTRODUCTION

Monogenetic parasites are the most common ectoparasites of fish in aquaculture systems. They are responsible for the loss of gill function, jeopardizing respiration and salt exchange with water, leading to fish mortality (Pavanelli et al., 2008; Godoi et al., 2012). Parasitized fish are more susceptible to infections since lesions serve as a pathway for bacterial and viral agents (Xu et al., 2007).

As a sustainable alternative to chemotherapy, herbal medicines and their by-products have been investigated to prevent and treat different diseases, especially those caused by parasites (Hashimoto et al., 2016; Bandeira et al., 2017; Raman, 2017; Silva et al., 2021). Herbal medicines can be applied orally through dietary supplementation. However, therapeutic baths are more frequently used against parasites (De Andrade et al., 2018; Silva et al., 2021).

Mint (*Mentha x villosa*) is a prominent plant used as an herbal medicine due to its anti-inflammatory, anti-hepatotoxic, anthelmintic and immunostimulant properties (Arruda et al., 2006). The use of its essential oil on *Schistosoma mansoni* worms resulted in decreased motility and, in higher concentrations, worm mortality (Matos-Rocha et al., 2013). On the other hand, *M. x villosa* hydrolate, a by-product of the distillation of the essential oil, exerted an ovicidal effect on bovine gastrointestinal nematodes in an *in-vitro* test (Nascimento et al., 2009), and it was already used as an antiparasitic for fish (Silva et al., 2021).

Although many studies have reported on alternative treatments for fish, few have evaluated the same protocol for different species. In the case of tilapias, studies are focused on the Nile tilapia (*Oreochromis niloticus*). Yet, other species of the genus *Oreochromis* are commercially important, including *Oreochromi aureus*, *Oreochromi mossambicus*, and *Oreochromi hornorum* that are reared worldwide, especially Asia and Africa (El-Sayed, 2019).

Therefore, this investigation aimed to evaluate the efficacy of the therapeutic bath with mint hydrolate in the control of monogeneans for four tilapia species (*O. niloticus*, *O. aureus*, *O. mossambicus*, and *O. hornorum*) reared in the same environment.

MATERIALS AND METHODS

This work was carried out at the Laboratório de Aquicultura (Laq), in the Instituto Federal Catarinense (IFC), Araquari Campus, Araquari (SC), Brazil. Fish were kept in ponds at Laq/IFC. This research was approved by the Animal Ethics Committee (Protocol No. 192/2017) of IFC. All species were kept in different ponds at LAq, and they were originated from the same laboratory.

Mentha x villosa hydrolate, a by-product of essential oil distillation, was obtained by hydrodistillation (Venskutonis et al., 1997) using the Clevenger apparatus, adapted to a 2,000-mL flask in which the mint leaves were placed together with 1,000 mL of distilled water according to Coradi et al. (2018). Fresh leaves of *M. x villosa* (150 g) were used for each distillation. The extraction time was 90 min, starting from the time of boiling. After the hydrolate (mixture of oil and water) was obtained, the separation of the essential oil was initiated using the organic solvent pentane (3 × 50 mL) in a separating funnel. After a few minutes of resting, the solution was filtered and concentrated on a rotary evaporator at 40°C, until the solvent volume was significantly reduced. The main compound was Carvone (Silva et al., 2021).

Fishpond

Tilapias (*O. niloticus*, *O. aureus*, *O. mossambicus*, and *O. hornorum*) used in this research were reared in a pond (32 m^3, 11.6 m × 5 m × 0.5 m) covered with geomembrane (8 mm). The pond had a daily water renewal rate of 5%. Fish were fed three times a day with commercial diet (Guabi, 32% crude protein). The stocking density was 12 $fish \cdot m^{-3}$, 100 per species. Water quality parameters were measured daily (8 h), with temperature ranging from 24.02 ~ 31.49°C, pH 6.85 ~ 7.33, dissolved oxygen above 3.36 $mg \cdot L^{-1}$, alkalinity above 23.01 mg $CaCO3 \cdot L^{-1}$, total ammonia bellow 0.07 $mg \cdot L^{-1}$, and nitrate bellow 21.72 $mg \cdot L^{-1}$.

Experimental design of bath treatment

After two months of rearing, sixty *O. niloticus* (212.72 ± 40.6 g and 21.6 ± 1.8 cm) were transferred to tanks (20 L) to carry out the experimental baths. The tanks were filled with water from the pond, and they were equipped with a constant aeration system. Fish were divided into six groups of ten per tank. Three tanks were submitted to therapeutic baths for 1 hour with *M. x villosa* hydrolate at concentrations of 20 $mL \cdot L^{-1}$ (Silva et al., 2021), and the other three tanks were used as a control group, whose fish were not exposed to the therapy, only submitted to the same handling procedures. This process was the same for all species: *O. aureus* (180.02 ± 37.5 g and 20.3 ± 1.7 cm), *O. mossambicus* (162.81 ± 44.3 g and 19.8 ± 1.8 cm), and *O. hornorum* (166.36 ± 43.4 g and 19.5 ± 1.6 cm). Each tank was equipped with aeration system (pumped air) with a stocking density of 0.5 fish·L^{-1}.

Parasitological analysis

After 1 hour of therapeutic bath, mucus was collected by craniocaudal scraping from both sides of the fish and stored in 4% buffered formalin for further counting. Then, the tilapia was anesthetized with eugenol (50 mg·L^{-1}) and euthanized by pithing of the spinal cord. The gills were removed and placed in Petri dishes containing the same maintenance water as the fish and were evaluated fresh immediately after collection under a stereomicroscope (Zeiss Stemi DV4), according to Jerônimo et al. (2011). For the study of the sclerotized parts, the Monogenea were mounted in Hoyer's medium (Eiras et al., 2006), and later identified as belonging to the family Dactylogyridae. Prevalence (P), mean intensity (MI) and mean abundance (MA) were determined following Bush et al. (1997). Efficacy was determined as Eq. 1:

$$E = MNPCG - MNPTG \times 100 / MNPCG \quad (1)$$

In which:
E = efficacy; MNPCG = the mean number of parasites in the control group; MNPGT = the mean number of parasites in the treated group (Dotta et al., 2015).
The same procedures were performed with the control group.

Statistical analysis

Data were first analyzed with Shapiro-Wilk's test for normality and then with Bartlett's test to verify the homogeneity of variance. All data were subjected to two-way (4 × 2; factor 1, species; factor 2, therapeutic bath) analysis of variance (ANOVA), and significant differences between treatments were analyzed using the Tukey's test (Statistica 8.0, StatSoft, Inc.). All tests were conducted at a 5%-level of significance.

RESULTS AND DISCUSSION

All tilapia species were infested with monogenean of the family Dactylogyridae with prevalence of 100% in mucus (Table 1) and gills (Table 2). Despite the 100% prevalence of monogeneans in the gills, the values of MI and MA were between 0.2 and 0.5, without showing a difference between tilapia species. In addition, there was no significant difference between treated and untreated tilapia with the therapeutic bath with mint hydrolate (Table 2). The absence of difference between treated and untreated fish may be related to the low presence of parasites in the gills beforehand.

Monogeneans cause several problems in freshwater fish culture in Brazil, whether to produce animal protein or for ornamental purposes (Mota Vicente et al., 2021; Steckert et al., 2021). Therapeutic baths are widely used for the prevention and/or treatment of diseases in fish culture, including salt, formalin, and essential oils (Hashimoto et al., 2016; García-Magaña et al., 2019; Roy et al., 2019).

The antiparasitic effect of essential oils for fish has already been confirmed (Da Costa et al., 2017; Soares et al., 2017), but few studies have reported the use of hydrolate as a phytotherapeutic (Nascimento et al., 2009; Silva et al., 2021). In this research, MA and MI in mucus were reduced after a 1-hour therapeutic bath in all tested species (Table 1). The efficacy of mint hydrolate was between 73.5% for *O. hornorum* and 84.5% for *O. niloticus* (Fig. 1). Silva et al. (2021) used the same hydrolate, but only for Nile tilapia, and verified efficacy near to 90%, corroborating our data. The use of *M. x villosa* hydrolate demonstrated better efficacy than *Mentha x piperita* when used in therapeutic bath (40 mg·L^{-1})– the second only had 41.63% of efficacy against monogeneans in Nile tilapia (*O. niloticus*) (Hashimoto et al., 2016).

Plants of the genus *Mentha* have several applications in aquaculture, such as food additive and antiparasitic agent and/or anesthetic (Hashimoto et al., 2016; Morcia et al., 2016; Silva et al., 2021). This may be related to the main component of mint hydrolate (Carvone), which is an enhancer of synthetic pesticides used in agriculture and veterinary medicine (Shah and Horsler, 2012), and it is known to be environmentally safe (Dos Santos Nascimento et al., 2020).

The interest in hydrolates usage increases. There is a growing need to gather knowledge on their composition and biological activity potential, and the development of drugs or alternative treatments for fish with the aim of significantly treating or controlling parasites is necessary for fish farming (Onaka et al., 2003). However, the effectiveness of its hydrolate by-product has still been neglected. For example, after a recent review on the use of hydrolate, only 10 studies reported antiparasitic effects, none of them in fish (Tavares et al., 2022). Silva et al. (2021) recorded the antiparasitic effect of the same hydrolate used in our work against monogeneans for tilapia. Furthermore, the dietary addition of *Curcuma longa* hydrolate to Nile tilapia showed benefits in the intestinal microbial community and increased the number of circulating lymphophytes, leading to a better physiological performance of Nile tilapia by maintaining fish homeostasis in rearing conditions (Pereira et al., 2020; 2021). This information corroborates our data, demonstrating the use of hydrolates as an important alternative to chemotherapy in aquaculture.

Table 1. Monogeneans indices (mean ± standard deviation) of mucus for four tilapia (*Oreochromis niloticus*, *O. aureus*, *O. mossambicus*, and *O. hornorum*) species after a 1-hour therapeutic bath of mint hydrolate.

Treatment		Parasitological index		
F1	**F2**	**Prevalence (%)**	**Mean intensity**	**Mean abundance**
O. niloticus	Control	100.0 ± 0.0^{Aa}	16.2 ± 3.9^{Aa}	16.2 ± 3.9^{Aa}
	Therapeutic bath	100.0 ± 0.0^{Aa}	2.5 ± 1.2^{Ab}	2.5 ± 1.2^{Ab}
O. mossambicus	Control	100.0 ± 0.0^{Aa}	14.3 ± 4.4^{Aa}	14.3 ± 4.4^{Aa}
	Therapeutic bath	100.0 ± 0.0^{Aa}	3.0 ± 1.4^{Ab}	3.0 ± 1.4^{Ab}
O. aureus	Control	100.0 ± 0.0^{Aa}	18.7 ± 5.1^{Aa}	18.7 ± 5.1^{Aa}
	Therapeutic bath	100.0 ± 0.0^{Aa}	3.6 ± 1.3^{Ab}	3.6 ± 1.3^{Ab}
O. hornorum	Control	100.0 ± 0.0^{Aa}	15.5 ± 5.6^{Aa}	15.5 ± 5.6^{Aa}
	Therapeutic bath	100.0 ± 0.0^{Aa}	4.1 ± 1.8^{Ab}	4.1 ± 1.8^{Ab}
Significance (p)				
Species (F1)		-	0.3930	0.3930
Therapeutic bath (F2)		-	0.0368	0.0368
F1xF2		-	0.3682	0.3682

*Different uppercase letters indicate significant differences among species, and different lowercase letters indicate significant differences between therapeutic bath and control in the bifactorial (two-way) analysis of variance and Tukey's tests ($p > 0.05$).

Table 2. Monogeneans indices (mean ± standard deviation) of gills for four tilapia (*Oreochromis niloticus*, *O. aureus*, *O. mossambicus*, and *O. hornorum*) species after a 1-hour therapeutic bath of mint hydrolate.

Treatment		Parasitological index		
F1	**F2**	**Prevalence (%)**	**Mean intensity**	**Mean abundance**
O. niloticus	Control	100.0 ± 0.0^{Aa}	0.5 ± 0.3^{Aa}	0.5 ± 0.3^{Aa}
	Therapeutic bath	100.0 ± 0.0^{Aa}	0.2 ± 0.4^{Aa}	0.2 ± 0.4^{Aa}
O. mossambicus	Control	100.0 ± 0.0^{Aa}	0.4 ± 0.4^{Aa}	0.4 ± 0.4^{Aa}
	Therapeutic bath	100.0 ± 0.0^{Aa}	0.2 ± 0.3^{Aa}	0.2 ± 0.3^{Aa}
O. aureus	Control	100.0 ± 0.0^{Aa}	0.4 ± 0.3^{Aa}	0.4 ± 0.3^{Aa}
	Therapeutic bath	100.0 ± 0.0^{Aa}	0.2 ± 0.4^{Aa}	0.2 ± 0.4^{Aa}
O. hornorum	Control	100.0 ± 0.0^{Aa}	0.5 ± 0.5^{Aa}	0.5 ± 0.5^{Aa}
	Therapeutic bath	100.0 ± 0.0^{Aa}	0.3 ± 0.3^{Aa}	0.3 ± 0.3^{Aa}
Significance (p)				
Species (F1)		-	0.4752	0.4752
Therapeutic bath (F2)		-	0.3295	0.3295
F1xF2		-	0.3903	0.3903

*Different uppercase letters indicate significant differences among species, and different lowercase letters indicate significant differences between therapeutic bath and control in the bifactorial (two-way) analysis of variance and Tukey's tests ($p > 0.05$).

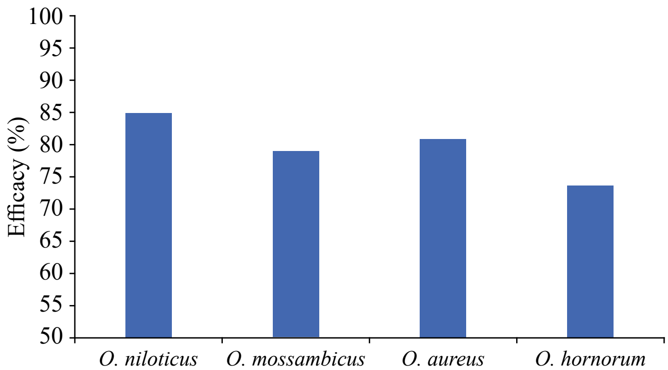

Figure 1. Efficacy of mint hydrolate bath as an antiparasitic agent against monogeneans in mucus for four tilapia (*Oreochromis niloticus*, *O. mossambicus*, *O. aureus*, and *O. hornorum*) species.

CONCLUSION

All tilapia species evaluated in this work demonstrated similar susceptibility to parasites when reared in the same environment. The use of mint therapeutic bath (1-hour treatment at concentrations of 20 mL·L^{-1}) demonstrated efficacy in the control of monogeneans in mucus for all evaluated species. Therefore, this protocol showed potential for use as a treatment in infestation by monogenetic parasites.

CONFLICT OF INTERESTS

Nothing to declare.

DATA AVAILABILITY STATEMENT

Data will be available upon request.

FUNDING

Not applicable.

AUTHORS' CONTRIBUTIONS

Conceptualization: Jatobá, A.; Stockhausen, L.; Silva, L.R.; Andrade, J.I.A.; **Data curation:** Stockhausen, L.; Silva, L.R.; **Formal Analysis:** Jatobá, A; **Investigation:** Jatobá, A.; Stockhausen, L.; Silva, L.R.; Andrade, J.I.A.; **Methodology:** Stockhausen, L.; Silva, L.R.; Andrade, J.I.A.; **Writing – original draft:** Jatobá, A.; Stockhausen, L.; Silva, L.R.; Andrade, J.I.A.; **Writing – review & editing:** Jatobá, A.; Andrade, J.I.A.

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