Aquaculture International (2022) 30:2245–2261
https://doi.org/10.1007/s10499-022-00900-z

# In vitro efficacy and tolerance of the essential oils of three species of the Lamiaceae family against monogeneans from the gills of *Piaractus brachypomus* from the Peruvian Amazon

**Anai Flores Gonzales[1] · Vanessa Mamani[1] · Manuel Pereyra[1] · Edwin Aguilar[1] · Patrick Delgado Mathews[2] · Marcos Tavares-Dias[3,4] · Christian Fernández-Méndez[5]**

Received: 17 December 2021 / Accepted: 11 May 2022 / Published online: 26 May 2022


**Abstract**
This study investigated the in vitro efficacy of the essential oil of *Minthostachys mollis*, *Origanum vulgare*, and *Salvia rosmarinus* against monogeneans from the gills of *Piaractus brachypomus* and the tolerance of this fish to these oils. In vitro concentrations of *M. mollis* (80, 200, 400, 600, 800, and 1000 mg $L^{-1}$), *O. vulgare*, *S. rosmarinus* (80, 200, 400, 600, 800, 1000, and 1500 mg $L^{-1}$) and two control groups (one containing only water from the growing tank and the other water from the tank + 70% alcohol) were evaluated at different exposure times. The concentrations were prepared at a proportion of 1:10 w/v with 70% alcohol, and after the in vitro tests, tolerance tests were carried out on fish with these oils. In the in vitro test, essential oil from *M. mollis*, *O. vulgare*, and *S. rosmarinus* showed dose-dependent efficacy against *Anacanthorus spathulatus*, *Anacanthorus penilabiatus*, and *Mymarothecium viatorum*. However, *M. mollis* caused the mortality of monogeneans in a shorter exposure time. In the two control groups using water from the growing tank and water from the growing tank + alcohol, the monogeneans showed similar behavior during the 6 h of exposure. In the tolerance tests with 80 mg $L^{-1}$ of essential oil from *M. mollis*, *O. vulgare*, and *S. rosmarinus* during 1 h of exposuse, it was observed that *P. brachypomus* showed irregular swimming and tolerance without causing death. Fish exposed to the other concentrations showed opercular acceleration, irregular swimming, lethargy, loss of mobility, and death after 1 h of exposure. Therefore, these essential oils can be tested in consecutive therapeutic baths at concentrations that have efficacy and exposure times less than sedation for the control and treatment of *P. brachypomus* monogeneans.

**Keywords** Aquaculture · Health · Medicinal plants · Phytotherapy

Handling Editor: Gavin Burnell

✉ Anai Flores Gonzales
agonzalesf@iiap.gob.pe

Extended author information available on the last page of the article

## Introduction

Global aquaculture is a highly productive activity that has grown rapidly in recent years (FAO 2020). In developing countries like Peru, aquaculture is complementing to national government's zero hunger policy, with the purpose of guaranteeing food security for communities and vulnerable populations. Continental aquaculture in Peru is growing and uses several species of fish, but especially *Piaractus brachypomus* (Cuvier 1818), one of the most important Amazonian species kept in captivity in several Latin American countries. This species adapts easily to confinement and balanced food (Negreiros and Tavares-Dias 2019).

Monogenean parasites affect several freshwater fish in productive systems (Valladão et al. 2016), and are considered pathogenic to hosts due to the lesions they cause in the gills and high infection levels, which can cause fish mortality and lead to economic losses to fish farmers (Tavares-Dias and Martins 2017). Thus, there is a need for the control and treatment of these fish parasites in cultivation. It has been reported that the gills of *P. brachypomus* can be infested by parasites of the Class Monogenean, such as *Anacanthorus spathulatus* Kritsky et al. (1979); *Anacanthorus penilabiatus* Boeger et al. (1995); *Mymarothecium viatorum* Boeger et al. (2002); and *Notozothecium janauachensis* Belmont-Jégu et al. (2004) (Oliveira and Tavares-Dias 2016; Negreiros and Tavares-Dias 2019). However, nowadays, alternative therapies such as the use of essential oils, which are environmentally friendly, non-accumulative, and biodegradable, have become popular (Valentim et al. 2018; Tavares-Dias 2018; Gonzales et al. 2019; 2020; Malheiros et al. 2020). Thus, essential oils obtained from plants have been used in aquaculture, as they have bioactive properties that act against monogeneans that parasitize fish (Soares et al. 2016; Barriga et al. 2020; Gonzales et al. 2020). In recent years, different essential oils have been used in therapeutic baths, added to feed or applied through nanoemulsions and nanocapsules to obtain greater effectiveness against monogeneans (Valentim et al. 2018; Tavares-Dias 2018; Gonzales et al. 2019; 2020; Malheiros et al. 2020). Herbal medicine such as essential oils has been shown to be an effective alternative for controlling and treating diseases caused by monogeneans that affect fish, with the purpose of improving productivity in fish farming, but there is still a lack of regulation on its use in the field, commercialization, and its impacts. Although more than 3000 essential oils obtained from medicinal plants are known, less than 0.4% of these oils have been evaluated in fish anti-parasitic studies (Tavares-Dias 2018).

In Peru, there are more than 19,500 species of medicinal plants registered, including native, endemic, and introduced species (Brako and Zarucchi 1993), and this great diversity is related to variations in the microclimates of the regions of the country. Most human uses of these plants involve traditional methods of combatting different diseases, through leaves and mixtures for the preparation of folk remedies (Rehecho et al. 2011).

The Lamiaceae family comprises a set of 12 subfamilies, 230 genera, and more than 7000 species, and is considered of great importance for its aromatic essential oils (Zhao et al. 2021). This family contains *Minthostachys mollis*, a sub-shrub native to Peru, *Origanum vulgare*, and *Salvia rosmarinus* which are cosmopolitan sub-shrubs. The leaves of these plants are used by the population for infusions to treat digestive diseases (gastritis, diarrhea, and colic), and as anthelmintic, diuretic, cold, aphrodisiac, stimulant, expectorant, and analgesic medicines. In addition, populations from Andean communities use leaves on food to protect against spoilage (Hammond et al. 1998; Rehecho et al.

2011). However, these essential oils have different major chemical constituents (Castro-Alayo et al. 2019; Leporini et al. 2020).

Based on the premise of use in ethnobotany and the bioactive capacities of *M. mollis*, *O. vulgare*, and *S. rosmarinus*, it is important to study the safe applicability of these medicinal plant species in aquaculture, since misuse can lead to toxicities and mortality of the fish, as there is little evidence of optimal concentrations that can be used in the control and treatment of parasitic diseases in fish. Thus, the present study aimed to evaluate the in vitro anti-parasitic efficacy of M. *mollis*, *O. vulgare*, and *S. rosmarinus* essential oils against *P. brachypomus* monogeneans, as well as the tolerance of this fish to such oils.

## Materials and methods

### Obtaining and chemical composition of essential oils

The essential oils of *M. mollis*, *O. vulgare*, and *S. rosmarinus* were obtained by steam distillation of dried leaves (Collected in the Cuzco region, La Convención Province) from a commercial supplier (Q'api, Tacna, Perú). The chemical composition of the essential oils was analyzed through qualitative and semi-quantitative analysis using gas chromatography coupled to liquid injection (GC–MS-Shimadzu QP210, Japan) mass spectrometry (Adams, 2007).

### Fish and monogenean maintenance

Juveniles of *P. brachypomus* were obtained through hormonal induction in the artificial reproduction of the Amazonian fish laboratory of the Peruvian Amazon Research Institute, Madre de Dios office. The post-larvae were kept in a 100-cm-deep excavated earthen pond at a density of 100 post-larvae per $m^3$ and fed with an extruded commercial feed (Aquatech, Naltech, Lima, Perú; proximal composition: 40% protein, 10% lipids, 12% ash; 10% moisture) and in the juvenile stage (from 7 cm approximately) were fed a commercial feed (Aquatech, Naltech, Lima, Perú) of 40% protein, 8% lipids, 10% ash, and 10% moisture. The excavated earthen ponds have a water replacement flow of 3 L/min from a water reservoir and by precipitation. The chemical and physical parameters of the water were monitored daily, twice a day (09:00–16:00 h), measuring dissolved oxygen ($3.5 \pm 0.5$ mg L), pH ($7.1 \pm 0.2$), and temperature ($31.3 \pm 1.0$ °C) with a multi-parameter meter (model HI98194, Hanna Instruments, USA) and photoperiod of 12 h light and 12 h dark. The juveniles used for the testing in this study were naturally parasitized on the gills by monogeneans. The monogenean class presents a simple and direct life cycle with a single host, they present an oncomiracid larvae that hatch from freely deposited aquatic eggs, and most monogeneans inhabit the skin and gills of the hosts (Kearn 1986).

### In vitro assays with *M. mollis, O. vulgare,* and *S. rosmarinus* and *P. brachypomus* monogeneans

To assess the anthelmintic efficacy and resistance of monogeneans to exposed concentrations of essential oils of *M. mollis, O. vulgare*, and *S. rosmarinus*, in vitro tests were carried out following the recommendations of Gonzales et al. (2019). A total of 25 juveniles

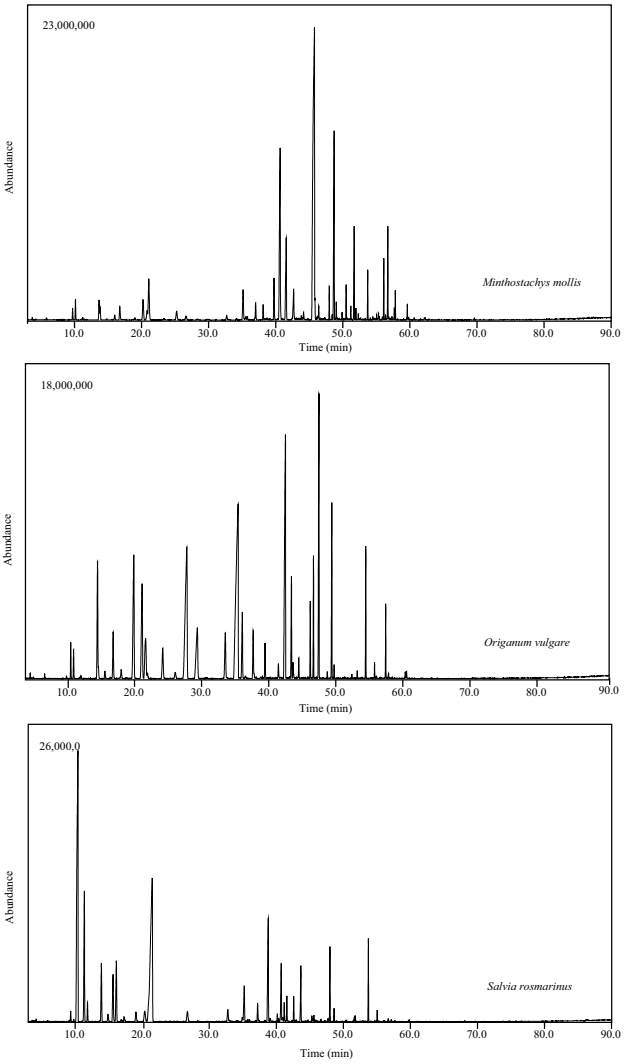


**Fig. 1** Chromatograms obtained from the GC–MS analysis of the essential oil of *Minthostachys mollis*, *Origanum vulgare*, and *Salvia rosmarinus*

of *P. brachypomus* ($52.8 \pm 13.4$ g and $14.1 \pm 1.3$ cm) were used for this assay and were naturally parasitized in the gills by monogeneans. The fish were sacrificed through cranial puncture resulting in instantaneous death and the extracted gills were then placed individually in Petri dishes. The gills were distributed into two control groups, one with water from the growing tank and the other with water from the tank + ethyl alcohol (70%), six different concentrations of *M. mollis* essential oil (80, 200, 400, 600, 800, and 1000 mg $L^{-1}$), and seven different concentrations of the essential oil of *O. vulgare* and *S. rosmarinus* (80, 200, 400, 600, 800, 1000, and 1500 mg $L^{-1}$), with three replicates for each treatment. The concentrations of each essential oil were prepared by diluting the essential oil at a proportion

**Table 1** Major chemical compounds of the essential oils of *Minthostachys mollis*, *Origanum vulgare*, *and Salvia rosmarinus*

| Area (%) | RT (min) | Compound name | Formula | CAS number |
|---|---|---|---|---|
| *Minthostachys mollis* | | | | |
| 36.76 | 45.774 | Pulegone | C10H16O | 89–82-7 |
| 13.43 | 40.659 | Menthone | C10H18O | 14,073–97-3 |
| 9.19 | 48.710 | Thymol | C10H14O | 89–83-8 |
| 4.94 | 41.564 | Isopulegone | C10H16O | 29,606–79-9 |
| 3.41 | 51.696 | 2- (1-Methylethylethylidene) cyclohexanone | C9H14O | 13,747–73-4 |
| 3.21 | 21.084 | Eucalyptol | C10H18O | 470–82-6 |
| 2.92 | 56.711 | Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethy | C15H24 | 3242–088 |
| 1.96 | 35.136 | Linalool | C10H18O | 78–70-6 |
| *Origanum vulgare* | | | | |
| 25.69 | 34.706 | Sabinene | C10H18O | 17,699–16-0 |
| 11.51 | 27.058 | γ-Terpinene | C10H16 | 99–85-4 |
| 11.43 | 41.760 | L-4-terpineol | C10H18O | 20,126–76-5 |
| 7.78 | 46.762 | Linalyl acetate | C12H20O2 | 115–95-7 |
| 6.27 | 19.152 | α-Terpinene | C10H16 | 99–86-5 |
| 4.87 | 20.394 | o-Cymene | C10H14 | 527–84-4 |
| 3.94 | 48.678 | Thymol | C10H14O | 89–83-8 |
| 2.58 | 42.658 | α-Terpinol | C10H18O | 98–55-5 |
| *Salvia rosmarinus* | | | | |
| 25.53 | 10.425 | α-Pinene | C10H16 | 7785–70-8 |
| 24.17 | 21.499 | Eucalyptol | C10H18O | 470–82-6 |
| 7.12 | 11.380 | Camphene | C10H16 | 79–92-5 |
| 6.41 | 38.799 | Camphor | C10H16O | 464–49-3 |
| 3.92 | 16.153 | Myrcene | C10H16 | 123–35-3 |
| 3.30 | 13.920 | β-Pinene | C10H16 | 18,172–67-3 |
| 3.13 | 15.655 | 3-Octanone | C8H16O | 106–68-3 |
| 3.07 | 40.731 | Borneol | C10H18O | 464–45-9 |

of 1:10 w/v in ethyl alcohol (70%), and weighed using an analytical balance (Ohaus® Pioneer® brand, model PA214). Subsequently, the oil concentrations were added to each of the three replicates (5.5 cm Petri dish) per treatment (Soares et al. 2016; Gonzales et al. 2020).

Using light stereoscopes with a field of view containing at least 20 monogeneans each, gill arches were selected for each repetition and, after submersion in different concentrations of essential oils, were observed every 5 min to quantify the number of alive or dead monogeneans. Dead parasites were those that had detached from the gill tissue and those adhered to the gill tissue that had completely lost mobility (Soares et al. 2016). The effectiveness of each treatment was then calculated (Zhang et al. 2014).

**Table 2** In vitro anthelmintic activity of six tested concentrations of the essential oil of *Minthostachys mollis* against monogeneans of *Piaractus brachypomus*

| Time of exposure | Treatments | No. of live parasites | Mortality (%) |
|---|---|---|---|
| 0 min | Water control | 22.6 ± 1.24 | 0 |
| 3 min | Water control | 22.6 ± 1.24 | 0 |
| 5 min | Water control | 22.6 ± 1.24 | 0 |
| 7 min | Water control | 22.6 ± 1.24 | 0 |
| 30 min | Water control | 22.6 ± 1.24 | 0 |
| 1 h | Water control | 22.3 ± 1.69 | 8.2 |
| 2 h | Water control | 21.0 ± 1.63 | 13.5 |
| 2 h 50 min | Water control | 20.3 ± 0.94 | 17.6 |
| 5 h | Water control | 15.6 ± 0.94 | 35.8 |
| 6 h 40 min | Water control | 0.0 ± 0.0 | 100 |
| 0 min | Alcohol control | 24.3 ± 1.24 | 0 |
| 3 min | Alcohol control | 24.3 ± 1.24 | 0 |
| 5 min | Alcohol control | 24.3 ± 1.24 | 0 |
| 7 min | Alcohol control | 24.3 ± 1.24 | 0 |
| 30 min | Alcohol control | 23.0 ± 1.63 | 5.3 |
| 1 h | Alcohol control | 21.3 ± 1.88 | 12.3 |
| 2 h | Alcohol control | 19.0 ± 1.41 | 21.8 |
| 2 h 50 min | Alcohol control | 18.0 ± 2.16 | 25.9 |
| 5 h | Alcohol control | 10.0 ± 2.44 | 58.8 |
| 6 h 20 min | Alcohol control | 0.0 ± 0.0 | 100 |
| 0 min | 80 mg $L^{-1}$ | 20.6 ± 0.57 | 0 |
| 3 min | 80 mg $L^{-1}$ | 20.6 ± 0.57 | 0 |
| 5 min | 80 mg $L^{-1}$ | 20.6 ± 0.57 | 0 |
| 7 min | 80 mg $L^{-1}$ | 19.6 ± 0.57 | 4.8 |
| 30 min | 80 mg $L^{-1}$ | 17.6 ± 0.57 | 24.6 |
| 1 h | 80 mg $L^{-1}$ | 13.0 ± 2.0 | 36.8 |
| 2 h | 80 mg $L^{-1}$ | 8.33 ± 2.51 | 59.5 |
| 2 h 50 min | 80 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 200 mg $L^{-1}$ | 20.3 ± 0.47 | 0 |
| 3 min | 200 mg $L^{-1}$ | 20.3 ± 0.47 | 0 |
| 5 min | 200 mg $L^{-1}$ | 19.3 ± 1.24 | 4.9 |
| 7 min | 200 mg $L^{-1}$ | 19.3 ± 1.24 | 4.9 |
| 30 min | 200 mg $L^{-1}$ | 15.3 ± 0.47 | 24.6 |
| 1 h | 200 mg $L^{-1}$ | 9.33 ± 2.05 | 54 |
| 2 h | 200 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 400 mg $L^{-1}$ | 20.3 ± 0.57 | 0 |
| 3 min | 400 mg $L^{-1}$ | 20.3 ± 0.57 | 0 |
| 5 min | 400 mg $L^{-1}$ | 17.3 ± 3.05 | 19.9 |
| 7 min | 400 mg $L^{-1}$ | 17.3 ± 3.05 | 19.9 |
| 30 min | 400 mg $L^{-1}$ | 8.66 ± 3.29 | 59.9 |
| 1 h | 400 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 600 mg $L^{-1}$ | 23.0 ± 0.0 | 0 |
| 3 min | 600 mg $L^{-1}$ | 13.0 ± 3.55 | 43.4 |
| 5 min | 600 mg $L^{-1}$ | 3.66 ± 3.09 | 84 |

**Table 2** (continued)

| Time of exposure | Treatments | No. of live parasites | Mortality (%) |
|---|---|---|---|
| 7 min | 600 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 800 mg $L^{-1}$ | 21.3 ± 0.94 | 0 |
| 3 min | 800 mg $L^{-1}$ | 5.66 ± 3.29 | 73.4 |
| 5 min | 800 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 1.000 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 3 min | 1.000 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |

### Test of *P. brachypomus* tolerance to *M. mollis*, *O. vulgare*, and *S. rosmarinus* essential oil

The tolerance test was carried out with the concentrations used in the in vitro tests, with the purpose of verifying which concentration was tolerable and safe without compromising fish survival, to be applied in therapeutic baths. Juveniles of *P. brachypomus* (31.5 ± 0.3 g and 11.3 ± 0.5 cm) were distributed in glass aquariums with a 20 L capacity. The essential oil treatments used were as follows: *M. mollis* (80, 200, 400, 600, 800, and 1000 mg $L^{-1}$), *O. vulgare*, and *S. rosmarinus* (80, 200, 400, 600, 800, 1000, and 1500 mg $L^{-1}$) with three repetitions for each essential oil concentration and five fish per repetition. Each essential oil was diluted in ethyl alcohol (70%) at a proportion of 1:10 w/v and placed in the water of the glass aquariums according to each concentration. Fish were exposed to each concentration for 1 h, and changes in fish behavior, swimming, and mortality were evaluated similar to other studies (Gonzales et al. 2020).

## Results

Chromatograms for the three EOs are presented in Fig. 1. The major chemical components of *M. mollis* are pulegone, menthone, and thymol, for *O. vulgare* γ-terpinene, L-4-terpineol, and linalyl acetate, and for *S. rosmarinus* α-pinene and eucalyptol. Sharing between them the compounds thymol and eucalyptol (Table 1).

The essential oil concentrations of *M. mollis*, *O. vulgare*, and *S. rosmarinus* showed efficacy in vitro against the monogeneans *A. spathulatus*, *A. penilabiatus*, and *M. viatorum*, but the exposure time varied among the species of these oils. At the highest concentration of *M. mollis* essential oil (1000 mg $L^{-1}$), total immobilization (100%) of the monogeneans occurred in just 3 min of exposure, while for *O. vulgare* and *S. rosmarinus*, a higher concentration was required (1500 mg $L^{-1}$) for total immobilization in 4 and 8 min respectively. The gills subjected to the alcohol control showed similar behavior to the water control group (Tables 2, 3, 4 and Fig. 2).

During the tolerance test in the exposure of essential oils of *M. mollis*, *O. vulgare*, and *S. rosmarinus* at a concentration of 80 mg $L^{-1}$, a tolerance was observed among fish, which only presented irregular swimming at 15, 17, and 20 min respectively (Table 5). All fish recovered after 60 min of exposure, with no record of mortality, but among the clinical signs, eye dilation was observed. At the following doses of essential oil exposure, fish initiated irregular swimming and lethargy between 2 and 7 min for *M. mollis* (1000–200 mg $L^{-1}$), between 2 and 12 min for *O. vulgare* (1500 to 200 mg $L^{-1}$), and between 4 and

**Table 3** In vitro anthelmintic activity of six tested concentrations of the essential oil of *Origanum vulgare* against monogeneans of *Piaractus brachypomus*

| Time of exposure | Treatments | No. of live parasites | Mortality (%) |
|---|---|---|---|
| 0 min | Water control | 22.6 ± 1.24 | 0 |
| 3 min | Water control | 22.6 ± 1.24 | 0 |
| 5 min | Water control | 22.6 ± 1.24 | 0 |
| 7 min | Water control | 22.6 ± 1.24 | 0 |
| 30 min | Water control | 22.6 ± 1.24 | 0 |
| 60 min | Water control | 22.3 ± 1.69 | 8.2 |
| 1 h | Water control | 21.0 ± 1.63 | 13.5 |
| 2 h 50 min | Water control | 20.3 ± 0.94 | 17.6 |
| 5 h | Water control | 15.6 ± 0.94 | 35.8 |
| 6 h 40 min | Water control | 0.0 ± 0.0 | 100 |
| 0 min | Alcohol control | 24.3 ± 1.24 | 0 |
| 3 min | Alcohol control | 24.3 ± 1.24 | 0 |
| 5 min | Alcohol control | 24.3 ± 1.24 | 0 |
| 7 min | Alcohol control | 24.3 ± 1.24 | 0 |
| 30 min | Alcohol control | 23.0 ± 1.63 | 5.3 |
| 1 h | Alcohol control | 21.3 ± 1.88 | 12.3 |
| 2 h | Alcohol control | 19.0 ± 1.41 | 21.8 |
| 2 h 50 min | Alcohol control | 18.0 ± 2.16 | 25.9 |
| 5 h | Alcohol control | 10.0 ± 2.44 | 58.8 |
| 6 h 20 min | Alcohol control | 0.0 ± 0.0 | 100 |
| 0 min | 80 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 4 min | 80 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 8 min | 80 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 25 min | 80 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 40 min | 80 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 1 h | 80 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 1 h 50 min | 80 mg $L^{-1}$ | 16.0 ± 0.8 | 20 |
| 3 h | 80 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 200 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 4 min | 200 mg $L^{-1}$ | 20.0 ± 0.1 | 0 |
| 8 min | 200 mg $L^{-1}$ | 20.0 ± 0.2 | 0 |
| 25 min | 200 mg $L^{-1}$ | 19.5 ± 0.5 | 2.5 |
| 40 min | 200 mg $L^{-1}$ | 18.5 ± 0.5 | 7.5 |
| 1 h | 200 mg $L^{-1}$ | 13.0 ± 2.0 | 35 |
| 1 h 50 min | 200 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 400 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 4 min | 400 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 8 min | 400 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 25 min | 400 mg $L^{-1}$ | 16.0 ± 1.0 | 20 |
| 40 min | 400 mg $L^{-1}$ | 7.0 ± 4.0 | 65 |
| 1 h | 400 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 600 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 4 min | 600 mg $L^{-1}$ | 17.0 ± 1.0 | 15 |
| 8 min | 600 mg $L^{-1}$ | 15.0 ± 1.0 | 25 |

**Table 3** (continued)

| Time of exposure | Treatments | No. of live parasites | Mortality (%) |
|---|---|---|---|
| 25 min | 600 mg $L^{-1}$ | 5.5 ± 1.5 | 72.5 |
| 40 min | 600 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 800 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 4 min | 800 mg $L^{-1}$ | 12.5 ± 3.5 | 37.5 |
| 8 min | 800 mg $L^{-1}$ | 7.5 ± 1.5 | 62.5 |
| 25 min | 800 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 1.000 mg $L^{-1}$ | 20 ± 0.0 | 0 |
| 4 min | 1.000 mg $L^{-1}$ | 2.5 ± 0.5 | 87.5 |
| 8 min | 1.000 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 1.500 mg $L^{-1}$ | 20.7 ± 0.5 | 0 |
| 4 min | 1.500 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |

13 min for *S. rosmarinus* (1500 to 200 mg $L^{-1}$). Loss of balance and mobility (anesthesia) in fish occurred between 3 and 11 min of exposure with *M. mollis*, between 3 and 14 min of exposure with *O. vulgare*, and between 6 and 15 min of exposure with *S. rosmarinus*. After the water change, there was no recovery of the fish, which is considered mortality, and body discoloration was observed in all doses higher than 200 mg $L^{-1}$.

## Discussion

Essential oils are complex hydrophobic and volatile aromatic mixtures that are synthesized in different parts of medicinal plants. They are known as secondary metabolites that are divided into two chemical classes: terpenoids and phenylpropanoids. The chemical composition, production, and yield of essential oils depend on several physiological, biochemical, environmental, genetic, and geographic factors (Sangwan et al. 2001; Ertas et al. 2015; Tavares-Dias 2018). Essential oils are widely used around the world in the areas of food, cosmetics, perfumery, and human medicine (Hernandes et al. 2017), but also in the cultivation of fish and other larger animals such as cattle and poultry, due to their bactericidal, anti-fungal, anthelmintic, acaricide, growth-promoting, anti-oxidant, and immunostimulant actions (Tavares-Dias 2018; Alimi et al. 2021; Ning et al. 2021). The chemical compounds obtained have been reported in another study of *M. mollis* essential oil as are

**Table 4** In vitro anthelmintic activity of six tested concentrations of the essential oil of *Salvia rosmarinus* against monogeneans of *Piaractus brachypomus*

| Time of exposure | Treatments | No. of live parasites | Mortality (%) |
|---|---|---|---|
| 0 min | Water control | 22.6 ± 1.24 | 0 |
| 3 min | Water control | 22.6 ± 1.24 | 0 |
| 5 min | Water control | 22.6 ± 1.24 | 0 |
| 7 min | Water control | 22.6 ± 1.24 | 0 |
| 30 min | Water control | 22.6 ± 1.24 | 0 |
| 1 h | Water control | 22.3 ± 1.69 | 8.2 |
| 2 h | Water control | 21.0 ± 1.63 | 13.5 |
| 2 h 50 min | Water control | 20.3 ± 0.94 | 17.6 |
| 5 h | Water control | 15.6 ± 0.94 | 35.8 |
| 6 h 40 min | Water control | 0.0 ± 0.0 | 100 |
| 0 min | Alcohol control | 24.3 ± 1.24 | 0 |
| 3 min | Alcohol control | 24.3 ± 1.24 | 0 |
| 5 min | Alcohol control | 24.3 ± 1.24 | 0 |
| 7 min | Alcohol control | 24.3 ± 1.24 | 0 |
| 30 min | Alcohol control | 23.0 ± 1.63 | 5.3 |
| 1 h | Alcohol control | 21.3 ± 1.88 | 12.3 |
| 2 h | Alcohol control | 19.0 ± 1.41 | 21.8 |
| 2 h 50 min | Alcohol control | 18.0 ± 2.16 | 25.9 |
| 5 h | Alcohol control | 10.0 ± 2.44 | 58.8 |
| 6 h 20 min | Alcohol control | 0.0 ± 0.0 | 100 |
| 0 min | 80 mg $L^{-1}$ | 23.5 ± 0.6 | 0 |
| 8 min | 80 mg $L^{-1}$ | 23.5 ± 0.6 | 0 |
| 20 min | 80 mg $L^{-1}$ | 23.5 ± 0.6 | 0 |
| 1 h 5 min | 80 mg $L^{-1}$ | 23.5 ± 0.6 | 0 |
| 1 h 15 min | 80 mg $L^{-1}$ | 21.3 ± 2.9 | 9.36 |
| 1 h 50 min | 80 mg $L^{-1}$ | 18.0 ± 0.0 | 23.4 |
| 2 h 55 min | 80 mg $L^{-1}$ | 4.0 ± 1.0 | 82.9 |
| 4 h | 80 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 200 mg $L^{-1}$ | 22.0 ± 0.0 | 0 |
| 8 min | 200 mg $L^{-1}$ | 22.0 ± 0.0 | 0 |
| 20 min | 200 mg $L^{-1}$ | 22.0 ± 0.0 | 0 |
| 1 h 5 min | 200 mg $L^{-1}$ | 22.0 ± 0.0 | 0 |
| 1 h 15 min | 200 mg $L^{-1}$ | 21.0 ± 1.0 | 4.54 |
| 1 h 50 min | 200 mg $L^{-1}$ | 16.0 ± 3.0 | 27.7 |
| 2 h 55 min | 200 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 400 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 8 min | 400 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 20 min | 400 mg $L^{-1}$ | 20.0 ± 0.0 | 0 |
| 1 h 5 min | 400 mg $L^{-1}$ | 12.0 ± 6.0 | 40 |
| 1 h 15 min | 400 mg $L^{-1}$ | 11.0 ± 6.0 | 45 |
| 1 h 50 min | 400 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 600 mg $L^{-1}$ | 22.7 ± 0.6 | 0 |
| 8 min | 600 mg $L^{-1}$ | 22.7 ± 0.6 | 0 |
| 20 min | 600 mg $L^{-1}$ | 20.5 ± 5.8 | 9.69 |

**Table 4** (continued)

| Time of exposure | Treatments | No. of live parasites | Mortality (%) |
|---|---|---|---|
| 1 h 5 min | 600 mg $L^{-1}$ | 3.5 ± 2.5 | 84.5 |
| 1 h 15 min | 600 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 800 mg $L^{-1}$ | 23.0 ± 2.0 | 0 |
| 8 min | 800 mg $L^{-1}$ | 22.5 ± 2.0 | 2.17 |
| 20 min | 800 mg $L^{-1}$ | 7.5 ± 0.5 | 67.3 |
| 1 h 5 min | 800 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 1.000 mg $L^{-1}$ | 21 ± 1.0 | 0 |
| 8 min | 1.000 mg $L^{-1}$ | 7.0 ± 0.0 | 66.6 |
| 20 min | 1.000 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |
| 0 min | 1.500 mg $L^{-1}$ | 20.7 ± 0.5 | 0 |
| 8 min | 1.500 mg $L^{-1}$ | 0.0 ± 0.0 | 100 |

β-phellandrene, D-limonene, linalool, α-pinene, pulegone, and L-menthone (Castro-Alayo et al. 2019), for *O. vulgare* are cis-β-terpineol, thymol, and L-4-terpineol (Carhuallanqui-Pérez et al. 2020), and for *S. rosmarinus* are 1,8-cineole, α-pinene, β-pinene, and camphor as main chemical compounds (Leporini et al. 2020). The chemical composition of essential oils can vary even in the same species depending on the time of collection, extraction method, and geographical location (Barra 2009). The essential oils of *M. mollis*, *O. vulgare*, and *S. rosmarinus* showed 100% in vitro efficacy against monogeneans, but this efficacy was dependent on concentration and exposure time. Other studies also reported dose-dependent effects for in vitro exposure on essential oils from *Cymbopogom citratus* (Gonzales et al. 2020), *Lippia grata* (Barriga et al. 2020), *Piper callosum*, *Piper aduncum*, and *Piper marginatum* (Alves et al. 2021). The *M. mollis* essential oil showed 100% efficacy within a few minutes against parasites at a lower concentration (1000 mg $L^{-1}$) compared to the essential oil of *O. vulgare* and *S. rosmarinus* (1500 mg $L^{-1}$). The in vitro efficacy of essential oils may differ among plants of the same genera due to their chemical composition (Barra 2009), as reported in in vitro assays with essential oil from *P. callosum*, *P. marginatum*, and *P. hispidum* (Alves et al. 2021). This anti-parasitic effectiveness is mainly related to the synergistic bioactive effects of the main chemical compounds of the essential oil species (Tavares-Dias 2018; Alves et al. 2021; Luz et al. 2021). Essential oils cause extensive damage and perforation of the integument, leading to death to the parasite and has been evidenced through scanning electron microscopy (Gonzales et al. 2020; Malheiros et al. 2020).

In the tolerance tests of the three essential oils tested, the concentration of 80 mg $L^{-1}$ did not cause sedation or mortality in fish during 1 h; however, this dose has very low effectiveness (0–36%) in 1 h, requiring more hours to reach 100%. The use of doses higher than 200 mg $L^{-1}$ in therapeutic baths has shown sedation in fish at different times (2 to 15 min) depending on the concentration. Similar results have been reported in other studies with essential oils for the control of monogeneans in fish (Barriga et al. 2020; Gonzales et al. 2020; Alves et al. 2021; Luz et al. 2021). Therefore, some essential oils may have limited use for therapeutic baths, due to the sedative action in high concentrations, which would hinder the control of parasites in fish, as they can compromise the survival of exposed fish (Malheiros et al. 2016; Soares et al. 2016). However,

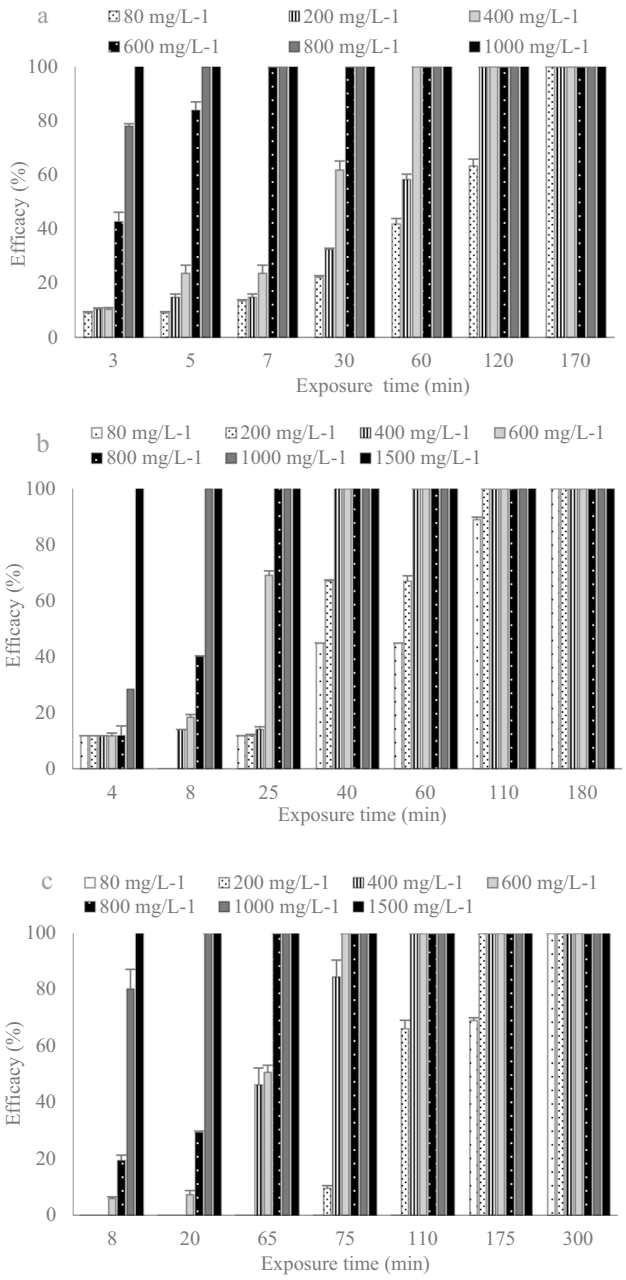


**Fig. 2** In vitro anthelmintic efficacy of the essential oil of *Minthostachys mollis* (**a**), *Origanum vulgare* (**b**), and *Salvia rosmarinus* (**c**) against monogeneans from the gills of *Piaractus brachypomus*

studies have evaluated the alternative of using repeated short baths with low doses to improve efficiency without affecting fish survival (Gonzales et al. 2020), as long as they present efficacy greater than 50% to be used in the field (Soares et al. 2016).

**Table 5** Tolerance of *Piaractus brachypomus* to different concentrations of essential oil after 1 h of exposure

| Essential oil species | Concentrations (mg $L^{-1}$) | Behavioral changes of fish (time) |
|---|---|---|
| *Minthostachys mollis* | 80 | Intensity of opercular beat (10 min); irregular swimming (15 min); water change and recovery of fish (60 min) |
| | 200 | Accelerated opercular beat (4 min); irregular swimming and lethargy (7 min); loss of equilibrium and mobility (11 min); water change, no recovery, and death (60 min) |
| | 400 | Accelerated opercular beat (2 min); irregular swimming and lethargy (5 min); loss of equilibrium and mobility (6 min); water change, no recovery, and death (60 min) |
| | 600 | Accelerated opercular beat and agitation (2 min); irregular swimming and lethargy (4 min); loss of equilibrium and mobility (5 min); water change, no recovery, and death (60 min) |
| | 800 | Accelerated opercular beat, agitation, and jumps (1 min); irregular swimming and lethargy (3 min); loss of equilibrium and mobility (4 min); water change, no recovery, and death (60 min) |
| | 1000 | Accelerated opercular beat, agitation, jumps, and convulsions (1 min); irregular swimming and lethargy (2 min); loss of equilibrium and mobility (3 min); water change, no recovery, and death (60 min) |
| *Origanum vulgare* | 80 | Intensity of opercular beat (13 min); irregular swimming (17 min); water change and recovery of fish (60 min) |
| | 200 | Accelerated opercular beat (10 min); irregular swimming and lethargy (12 min); loss of equilibrium and mobility (14 min); water change, no recovery, and death (60 min) |
| | 400 | Accelerated opercular beat (6 min); irregular swimming and lethargy (9 min); loss of equilibrium and mobility (11 min); water change, no recovery, and death (60 min) |
| | 600 | Accelerated opercular beat (3 min); irregular swimming and lethargy (5 min); loss of equilibrium and mobility (8 min); water change, no recovery, and death (60 min) |
| | 800 | Accelerated opercular beat and agitation (2 min); irregular swimming and lethargy (3 min); loss of equilibrium and mobility (5 min); water change, no recovery, and death (60 min) |
| | 1000 | Accelerated opercular beat, agitation, and jumps (2 min); irregular swimming and lethargy (3 min); loss of equilibrium and mobility (4 min); water change, no recovery, and death (60 min) |
| | 1500 | Accelerated opercular beat, agitation, jumps, and convulsions (1 min); irregular swimming and lethargy (2 min); loss of equilibrium and mobility (3 min); water change, no recovery, and death (60 min) |

**Table 5** (continued)

| Essential oil species | Concentrations (mg $L^{-1}$) | Behavioral changes of fish (time) |
|---|---|---|
| *Salvia rosmarinus* | 80 | Intensity of opercular beat (15 min); irregular swimming (20 min); water change and recovery of fish (60 min) |
| | 200 | Accelerated opercular beat (12 min); irregular swimming and lethargy (13 min); loss of equilibrium and mobility (15 min); water change, no recovery, and death (60 min) |
| | 400 | Accelerated opercular beat (7 min); irregular swimming and lethargy (10 min); loss of equilibrium and mobility (13 min); water change, no recovery, and death (60 min) |
| | 600 | Accelerated opercular beat (5 min); irregular swimming and lethargy (8 min); loss of equilibrium and mobility (12 min); water change, no recovery, and death (60 min) |
| | 800 | Accelerated opercular beat and agitation (4 min); irregular swimming and lethargy (8 min); loss of equilibrium and mobility (10 min); water change, no recovery, and death (60 min) |
| | 1000 | Accelerated opercular beat, agitation, and jumps (2 min); irregular swimming and lethargy (5 min); loss of equilibrium and mobility (8 min); water change, no recovery, and death (60 min) |
| | 1500 | Accelerated opercular beat, agitation, jumps, and convulsions (2 min); irregular swimming and lethargy (4 min); loss of equilibrium and mobility (6 min); water change, no recovery, and death (60 min) |

Essential oils emerge as an alternative to treat monogenean diseases as opposed to widely used chemicals such as formalin, which is highly carcinogenic and allergenic in humans, toxic to fish, and environmentally hazardous due to residues (Hoque et al. 2016; Tavares-Dias 2021). However, further experiments should be conducted with other doses of essential oil or using new technologies such as nanoemulsion to reduce the toxicity and quantity of essential oils without losing their effectiveness against fish parasites as a sustainable strategy in aquaculture.

**Author contribution** Methodology, validation, conceptualization, research, manuscript writing, supervision, APFG; methodology, conceptualization, VM, MP, and EA; critical review of manuscript and conceptualization, PMD and MTD; methodology design, validation, manuscript review, funding acquisition, CFM. All authors have read and agreed to the published version of the manuscript.

**Funding** This research was funded by Concytec—Banco Mundial "Mejoramiento y Ampliación de los Servicios del Sistema Nacional de Ciencia Tecnología e Innovación Tecnológica" 8682-PE, through its executing unit ProCiencia (grant number 116–2018- FONDECYT-BM-IADT-SE) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasil) through the productivity research grant awarded to Dr. M. Tavares-Dias (Grant No. 303013/2015–0).

**Data availability** All data generated or analyzed during this study are included in this published article.

**Code availability** Not applicable.

## Declarations

**Ethics approval** Experimental procedures with animals were carried out in accordance with Peruvian legislation for animal protection and welfare in compliance with the protocol of the ethics committee for the use of animals in acts of experimentation, research, and teaching (30407/2016).

**Conflict of interest** The authors declare no competing interests.

## References

Adams RP (2007) Identification of essential oils components by gas chromatography/mass 402 spectrometry, 4th edn. Allured Publishing Corporation, Carol Stream, IL, USA, p 808

Alimi D, Hajri A, Jallouli S, Sebai H (2021) *In vitro* acaricidal activity of essential oil and crude extracts of *Laurus nobilis*, (Lauraceae) grown in Tunisia, against arthropod ectoparasites of livestock and poultry: *Hyalomma scupense* and *Dermanyssus gallinae*. Vet Parasitol 298:109507

Alves CMG, Nogueira JN, Luz JGR, Chaves FCM, Tavares-Dias M (2021) Essential oil of *Piper callosum, Piper hispidum* and *Piper marginatum* (Piperaceae) possesses *in vitro* efficacy against monogeneans of *Colossoma macropomum* (tambaqui). Aquac Res 00:1–10

Barra A (2009) Factors affecting chemical variability of essential oils: a review of recent developments. Nat Prod Commun 4:1147–1154

Barriga IB, Gonzales APPF, Renato A, Brasiliense P, Neoob K, Castro C (2020) Essential oil of *Lippia grata* (Verbenaceae) is effective in the control of monogenean infections in *Colossoma macropomum* gills, a large Serrasalmidae fish from Amazon. Aquac Res 51:1–9

Belmont-Jégu E, Domingues MV, Martins ML (2004) Notozothecium janauachensis n. sp. (Monogenoidea:Dactylogyridae) from wild and cultured tambaqui, Colossoma macropomum (Teleostei:Characidae: Serrasalminae) in Brazil. Zootaxa 736:1–8

Boeger WA, Husak WS, Martins ML (1995) Neotropical Monogenoidea. 25. Anacanthorus penilabiatus n. sp. (Dactylogyridae, Anacanthorinae) from Piaractus mesopotamicus (Osteichthyes, Serrasalmidae), cultivated in the state of Sao Paulo Brazil. Memorias do Instituto Oswaldo Cruz 90:699–701

Boeger WA, Piasecki W, Sobecka E (2002) Neotropical monogenoidea. 44. Mymarothecium viatorum sp. n. (Ancyrocephalinae) from the gills of Piaractus brachypomus (Serrasalmidae, Teleostei) captured in a warm-water canal of a power plant in Szczecin, Poland. Acta Ichthyol Piscat 32:157–162

Brako L, Zarucchi J (1993) Catalogue of the Flowering Plants and Gymnosperms of Peru. Crosby MR (ed), vol 45. Missouri Botanical Garden, Saint Louis

Carhuallanqui-Pérez A, Salazar-Salvatierra ME, Delgado DR (2020) Efecto antimicrobiano del aceite esencial de Orégano frente a *Listeria monocytogenes* y *Staphylococcus aureus*. Rev Investig Altoandin 22:25–33

Castro-Alayo EM, Chávez-Quintana SG, Auquiñivín-Silva EA, Fernández-Jeri AB, Acha-De la Cruz O, Rodríguez-Hamamura N, Olivas-Orozco GI, Sepúlveda-Ahumada DR (2019) Essential oils of native plants from Peru: effect of the place of cultivation on the physicochemical characteristics and antioxidant activity. Scientia Agropecuaria 10:479–487

Cuvier GL (1818) Sur les Poissons du sous-genre Mylètes. In: Memoires du Muséum d'Histoire Naturelle. Chez Jules Renouard et Comp, París, Francia, pp 444–456

Ertas A, Boga M, Yilmaz MA, Yesil Y, Tel G, Temel H, Hasimi N, Gazioglu I, Ozturk M, Ugurlu P (2015) A detailed study on the chemical and biological profiles of essential oil and methanol extract of *Thymus nummularius* (Anzer tea): rosmarinic acid. Ind Crops Prod 67:336–345

FAO (2020) El estado mundial de la pesca y la acuicultura 2020. La sostenibilidad en acción. Roma. https://doi.org/10.4060/ca9229es

Gonzales AF, Santos GG, Tavares M (2019) Anthelminthic potential of the *Ficus insipida* latex on monogeneans of *Colossoma macropomum* (Serrasalmidae), a medicinal plant from the Amazon. Acta Parasitol 64:927–931

Gonzales APPF, Yoshioka ETO, Mathews PD, Mertins O, Chaves FCM, Videira MN, Tavares-Dias M (2020) Anthelminthic efficacy of *Cymbopogon citratus* essential oil (Poaceae) against monogenean parasites of *Colossoma macropomum* (Serrasalmidae), and blood and histopathological effects. Aquaculture 528:735500

Hammond GB, Fernández ID, Villegas LF, Vaisberg AJ (1998) A survey of traditional medicinal plants from the Callejon de Huaylas, Department of Ancash, Peru. J Ethnopharmacol 61:17–30

Hernandes C, Pina ES, Taleb-Contini SH, Bertoni BW, Cestari IM, Espanha LG, Varanda EA, Camilo KFB, Martinez EZ, França SC, Pereira AMS (2017) *Lippia origanoides* essential oil: an efficient and safe alternative to preserve food, cosmetic and pharmaceutical products. J Appl Microbiol 122:900–910

Hoque MS, Jacxsens L, De Meulenaer B, Alam AN (2016) Quantitative risk assessment for formalin treatment in fish preservation: food safety concern in local market of Bangladesh. Procedia Food Sci 6:151–158

Kearn GC (1986) The eggs of monogeneans. Adv Parasitol 25:175–273

Kritsky DC, Thatcher VE, Kayton RJ (1979) Neotropical Monogenoidea. 2. The Anacanthorinae Price, 1967, with the proposal of four new species of Anacanthorus Mizelle and Price, 1965 from Amazonian fishes. Acta Amaz 9:355–361

Leporini M, Bonesi M, Loizzo MR, Passalacqua NG, Tundis R (2020) The essential oil of *Salvia rosmarinus* spenn. from Italy as a source of health-promoting compounds: chemical profile and antioxidant and cholinesterase inhibitory activity. Plants 9:1–13

Luz JGR, Nogueira JN, Alves CMG, Videira MN, Canuto KM, Castro KNC, Tavares-Dias M (2021) Essential oil of *Alpinia zerumbet* (Zingiberaceae) has anthelmintic efficacy against monogenean of *Colossoma macropomum* (Characiformes: Serrasalmidae). Aquac Res 00:1–10

Malheiros DF, Maciel PO, Videira MN, 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

Malheiros DFI, Sarquis R, Ferreira IM, Mathews PD, Mertins O, Tavares-Dias M (2020) Nanoemulsions with oleoresin of *Copaifera reticulata* (Leguminosae) improve anthelmintic efficacy in the control of monogenean parasites when compared to oleoresin without nanoformulation. J Fish Dis 43:687–695

Negreiros LP, Tavares-Dias (2019) Parasites in farmed *Piaractus brachypomus* (Serrasalmidae) in the state of acre, western Brazilian Amazonia. Acta Amaz 49:294–298

Ning L, Zhang X, Zhang D, Hu Y, Li Y (2021) The benefits of blend essential oil for GIFT tilapia on the digestion, antioxidant, and muscle quality during cold storage. Aquaculture 533:736097

Oliveira MSB, Tavares-Dias M (2016) Comunidades de parasitos metazoários em *Piaractus brachypomus* (Pisces, Serrasalmidae) no baixo Rio Amazonas, Brasil. Rev Bras Parasitol Vet 25:151–157

Rehecho S, Uriarte-Pueyo I, Calvo J, Vivas LA, Calvo MI (2011) Ethnopharmacological survey of medicinal plants in Nor-Yauyos, a part of the Landscape Reserve Nor-Yauyos-Cochas, Peru. J Ethnopharmacol 133:75–85

Sangwan NS, Farooqi AH, Shabih F, Sangwan RS (2001) Regulation of essential oil production in plant. Plant Growth Regul 34:3–21

Soares BVL, Neves R, Oliveira MSB, Chaves FCM, Dias MKR, Chagas EC, 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

Tavares-Dias M (2018) Current knowledge on use of essential oils as alternative treatment against fish parasites. Aquat Living Resour 31:13

Tavares-Dias M (2021) Toxicity, physiological, histopathological and antiparasitic effects of the formalin, a chemotherapeutic of fish aquaculture. Aquac Res 52:1803–1823

Tavares-Dias M, Martins ML (2017) An overall estimation of losses caused by diseases in the Brazilian fish farms. J Parasit Dis 41:913–918

Valentim DSS, Duarte JL, Oliveira AEMFM, Cruz RAS, Carvalho JCT, Solans C, Fernandes CP, Tavares-Dias M (2018) Effects of a nanoemulsion with *Copaifera officinalis* oleoresin against monogenean parasites of *Colossoma macropomum*: A Neotropical Serrasalmidae. J Fish Dis 41:1041–1048

Valladão GMR, Gallani SU, Ikefuti CV, da Cruz C, Levy-Pereira N, Rodrigues MVN, Pilarski F (2016) Essential oils to control ichthyophthiriasis in pacu, *Piaractus mesopotamicus* (Holmberg): special emphasis on treatment with *Melaleuca alternifolia*. J Fish Dis 39:1143–1152

Zhang XP, Li WX, Ai TS, Zou H, Wu SG, Wang GT (2014) The efficacy of four common anthelmintic drugs and traditional Chinese medicinal plant extracts to control *Dactylogyrus vastator* (Monogenea). Aquaculture 420–421:302–307

Zhao F, Chen YP, Salmaki Y, Drew BT, Wilson TC, Scheen AC, Celep F, Bräuchler C, Bendiksby M, Wang Q, Min DZ, Peng H, Olmstead RG, Li B, Xiang CL (2021) An updated tribal classification of Lamiaceae based on plastome phylogenomics. BMC Biol 19:1–27



## Authors and Affiliations

**Anai Flores Gonzales[1] · Vanessa Mamani[1] · Manuel Pereyra[1] · Edwin Aguilar[1] · Patrick Delgado Mathews[2] · Marcos Tavares-Dias[3,4] · Christian Fernández-Méndez[5]**

[1] Institute of Research the Peruvian Amazon (IIAP, AQUAREC), 17000 Puerto Maldonado, Peru

[2] Department of Zoology, Institute of Biosciences, University of São Paulo, São Paulo 05508–090, Brazil

[3] Embrapa Amapá, Macapá, AP 68903-419, Brazil

[4] Postgraduate Program On Tropical Biodiversity (PPGBIO), Federal University of Amapá (UNIFAP), Macapá, AP 68903-419, Brazil

[5] Institute of Research the Peruvian Amazon (IIAP, AQUAREC), Iquitos 16000, Peru