sciendo

DOI 10.2478/helm-2023-0019

HELMINTHOLOGIA, 60, 2: 125 – 133, 2023

Anthelmintic effects of peppermint (*Mentha piperita*), lemon (*Citrus limon*), and tea tree (*Melaleuca alternifolia*) essential oils against Monogenean parasite (*Dactylogyrus* sp.) on carp (*Cyprinus carpio*)

B. HARMANSA YILMAZ[1,*], H. YAVUZCAN YILDIZ[1]

[1]Ankara University, Faculty of Agriculture, Department of Aquaculture and Fisheries, Ankara, Turkey,
*E-mail: *bilgenurharmansah@gmail.com*

Article info

Received February 2, 2023
Accepted May 30, 2023

Summary

Dactylogyrus sp. (Monogenea) is one of the most dangerous pathogens causing parasitic infections in carp (*Cyprinus carpio*) and other freshwater fish. Due to the adverse effects of conventional chemical treatments on the environment and fish, the use of herbal products in aquaculture against various diseases has increased. In the present study, anthelmintic effects of peppermint (*Mentha piperita*), lemon (*Citrus limon*), and tea tree (*Melaleuca alternifolia*) essential oils against *Dactylogyrus* sp. found on the gills of carp were studied using *in vitro* and *in vivo* experiments. In *in vitro* experiments, 1, 2.5, 5, and 10 µl/ml concentrations of these essential oils were tested on the parasites and cumulative mortalities were observed to reach 100 % after treatment with peppermint, lemon, and tea tree essential oils of 1 µl/ml concentration in approximately 15, 10 and 2 min, respectively. The *in vitro* experiments demonstrated that the cumulative mortality of the parasites increased with essential oil concentration and exposure duration. As the concentration of essential oil used increased, the observed mean time to death of parasites decreased. In *in vivo* experiments, median effective concentrations (EC50) as assessed by *in vitro* tests for peppermint and lemon essential oils in 5 min exposure and EC50 concentration for tea tree essential oil in 2 min exposure was applied on fish as a single bath and there was a significant decrease in the mean parasite intensities ($p<0.05$). Antiparasitic efficacies of peppermint, lemon, and tea tree essential oils were determined as 28.23 %, 30.95 %, and 35.31 %, respectively. The tea tree oil was the most effective and peppermint oil was the least effective in both *in vitro* and *in vivo* experiments. All three herbal essential oils tested in this study have weak antiparasitic potential against monogenean infections in fish.
Keywords: antiparasitic effects; herbal essential oils; *Cyprinus carpio; Dactylogyrus* sp.

Introduction

The aquaculture industry has grown significantly in the world from 17.3 million tons of average annual production in the 1990s to 122.6 million tons in 2020. Carp is an economically important species in the industry and in 2020 common carp was ranked fourth worldwide in total finfish production in inland aquaculture (FAO, 2022). The rapid increase in production to meet multiplying demand has elevated disease risks by increasing stress factors such as suboptimal water quality, inadequate nutrition, and higher stocking density in aquaculture conditions. Ectoparasitic infections, particularly by monogeneans, have resulted in significantly greater losses in the fish culture industry (Ernst *et al*., 2002; Shinn *et al*., 2015).

* – corresponding author

Monogeneans are a group of parasitic flatworms that are commonly found on the skin or the gills of fish. These parasites are known for their host specificity, which means they tend to infect only one or a few closely related species of fish. Host specificity helps to prevent competition with other monogenean species that infect different fish hosts. Their short and direct life cycle induces the rapid invasion in fish gills or skin (Ernst *et al*., 2002). One of the Monogenean ectoparasites, the *Dactylogyrus* species exhibits hermaphroditism and oviparity. Their free-swimming ciliated larvae (oncomiracidia) emerge directly from eggs and are capable of infecting fish without the need for an intermediary host (Zoral *et al*., 2017). *Dactylogyrus* sp. causes serious damage in fish by triggering pathological changes that inhibit gas exchange in gills resulting in appetite loss, low growth performance, and high mortality rates (Obiekezie & Taege, 1991). In tissues damaged by parasites, secondary infections due to bacterial and fungal pathogens are also common (Reed *et al*., 2009).

A common treatment technique has been the application of chemicals like formalin, copper sulfate, potassium permanganate, and hydrogen peroxide on fish by the bath. While these chemicals can be effective, their use can negatively impact water quality, and the toxins they leave behind in fish pose risks to human health (Ling *et al*., 2015; Hashimoto *et al*., 2016; Soares *et al*., 2016; Soares *et al*., 2017a, b). For these reasons, the use of chemicals against fish diseases has been restricted in many countries (Ling *et al*., 2015). For example, inside the European Union, some of the chemical therapeutics such as malachite green, or methylene blue are already banned (Lieke *et al*., 2020). The research of alternative treatments for parasitic infections in fish has been prompted by these problems (Lieke *et al*., 2020; Yildiz & Bekcan, 2020; Phan *et al*., 2021).

Plant essential oils have been gaining interest in aquaculture as a natural alternative to antibiotics and other synthetic drugs for controlling diseases and improving growth performance in farmed aquatic animals. Essential oils are volatile, aromatic compounds extracted from different parts of plants, such as leaves, flowers, and fruits, that possess diverse biological activities, including antimicrobial, antiviral, antiparasitic, antioxidant, and immunostimulatory properties. Over the past few years, there has been an increasing interest in searching alternative treatment options for various fish parasites, as well as studying the cytotoxic properties of different herbal products such as plant extracts, essential oils, and bioactive metabolites derived from diverse plant sources. Some of these studies have yielded promising results with regard to their antiparasitic effects (Wang *et al*., 2011; Wu *et al*., 2011; Lu *et al*., 2012; Ramudu & Dash, 2013; Valladao *et al*., 2015; Tavares-Dias, 2018). Thus, herbal treatment emerged as having potential as an innovative method against various parasites in aquaculture (Soares & Tavares-Dias, 2013; Soares *et al*., 2016; Soares *et al*., 2017a, b).

Of the plants tested for their antiparasitic potential in this study, peppermint is used in medicine as an antimicrobial and an antioxidant. The main component of its essential oil is menthol (Kumar *et al*., 2012; Freire *et al*., 2012; Tsai *et al*., 2013). Lemon essential oil's antibacterial, antioxidant, and anticancer properties are due to the phenolic compounds it contains, especially limonin (Bulfon *et al*., 2015). Tea tree is an Australian plant containing the main metabolite of terpinen-4-ol and has a wide range of antiseptic, antimicrobial, anti-inflammatory, and antiparasitic activity (Thomsen *et al*., 2011). Peppermint and lemon essential oil have not been tested for their anti-helminthic activities in fish before to our knowledge. Tea tree oil has been tested on *Gyrodactylus* spp. infection of the three-spined stickleback *Gasterosteus aculeatus* (Steverding *et al*., 2005).

Phytotherapeutic antiparasitic properties of various essential oils have been evaluated against diverse species of monogeneans. The effectiveness of these treatments varied between 0 to 100 %, depending on the specific essential oil and therapeutic approach utilized. Ling *et al* (2015) reported that cinnamic acid and cinnamaldehyde were 100 % effective against *D.intermedius* in *Carassius auratus*. On the other hand, Soares *et al* (2017a) showed that the essential oils (EOs) extracted from *Lippia sidoides* were ineffective against *Anacanthorus spathulatus* in *Colossoma macropomum*. Numerous studies have indicated that essential oils may have the potential to treat *Dactylogyrus* species (Monogeneans) in aquaculture (Ling *et al*., 2015; Luo *et al*., 2016; Wang *et al*., 2011; Wu *et al*., 2011; Yao *et al*., 2011; Phan *et al*., 2021). Nevertheless, it is surprising to note that only a limited number of essential oils have been extensively studied and subjected to controlled experimentation to demonstrate their anthelmintic activity, either through *in vitro* assays or through therapeutic baths (*in vivo* assays) for parasitized hosts in aquaculture (Tavares-Dias, 2018). Thus, to the best of our knowledge, the antiparasitic activity of peppermint, lemon, and tea tree essential oils against fish monogenean, *Dactylogyrus* sp. has not been reported so far. Therefore, the present study attempted to investigate the anthelmintic activity of essential oils of peppermint, lemon, and tea tree against the monogenean parasite *(Dactylogyrus* sp.) on carp.

Materials And Methods

Fish and parasites

Carp weighing 50 – 100 g in the aquaponic system in Ankara University, Department of Fisheries and Aquaculture were used as fish material in the research. The study was carried out between March 2020 and September 2020 at Ankara University, Department of Fisheries and Aquaculture, Fish Health Laboratory. During the research, a total of 40 fish were used. Fish were placed in 2 fiber tanks (80x60x50 cm) (20 fish in each tank) ventilated by a dry air motor. The water temperature was maintained at 20 – 22 °C, dissolved oxygen at 5.50 – 5.97 mg/L, and pH at 6.97 – 7. Fish were fed with commercial feed containing 45 % raw protein at a 2 % total body weight ratio.

Table 1. *In vitro* cumulative mortality of *Dactylogyrus* sp. exposed to DMSO solution.

Concentration	Cumulative Mortality (%)											
	1 min	2 min	3 min	4 min	5 min	6 min	7 min	8 min	9 min	10 min	15 min	20 min
1/10	60	100	100	100	100	100	100	100	100	100	100	100
1/20	0	70	90	100	100	100	100	100	100	100	100	100
1/30	0	0	50	70	100	100	100	100	100	100	100	100
1/40	0	0	0	0	0	0	0	0	0	20	30	50
1/50	0	0	0	0	0	0	0	0	0	0	0	0

For *in vitro* analysis, adult parasites were collected from the gills of heavily infected carp. Carp were anesthetized in 25 mg/l concentration of clove oil solution and ectoparasite specimens were collected by light scraping from the gill mucus of carp as previously described by Yildiz and Bekcan (2020). Samples taken from the gill mucus were taken on a slide and examined under a binocular microscope. During the examination of the samples for parasites, principles reported by Lom and Dykova (1992) were applied. *Dactylogyrus* sp. was identified by observing four eye spots and a pair of hooks (Malmberg, 1970; Bruno *et al*., 2006).

Essential Oils

In the study, the antiparasitic effect of peppermint (main bioactive component: menthol), lemon (main bioactive component: limonene), and tea tree essential oils (main bioactive component: terpinen-4-ol) were investigated. Essential oils were obtained from the spice sellers (trade name: Unyazici) in Ankara. Since essential oils are not soluble in water dimethyl sulfoxide (DMSO) was used as a solvent.

In vitro determination of the antiparasitic effect of essential oils on Dactylogyrus sp.

In the *in vitro* experiments, during which the lethal effect of essential oils on *Dactylogyrus* sp. was examined, parasites collected from the gills were counted and then subjected to peppermint, lemon, and tea tree essential oils with 10, 5, 2.5, and 1 µl/ml concentrations between 1 and 15 min. In the experiments, each essential oil concentration was tested on 10 parasites. Parasites were examined continuously for their motility and shrinkage under the microscope and the times to nonmotility were recorded. Parasites that did not move and respond when touched with a needle were reported as dead. In the control group, parasites were exposed to DMSO, the solvent of essential oils. Since DMSO itself can have a lethal effect on the parasite, various concentrations of DMSO have been tested before starting the experiments to discard the possible effects of DMSO. The concentration of DMSO and water at a ratio of 1:50 has been determined to have no effect on the parasites (Table 1).

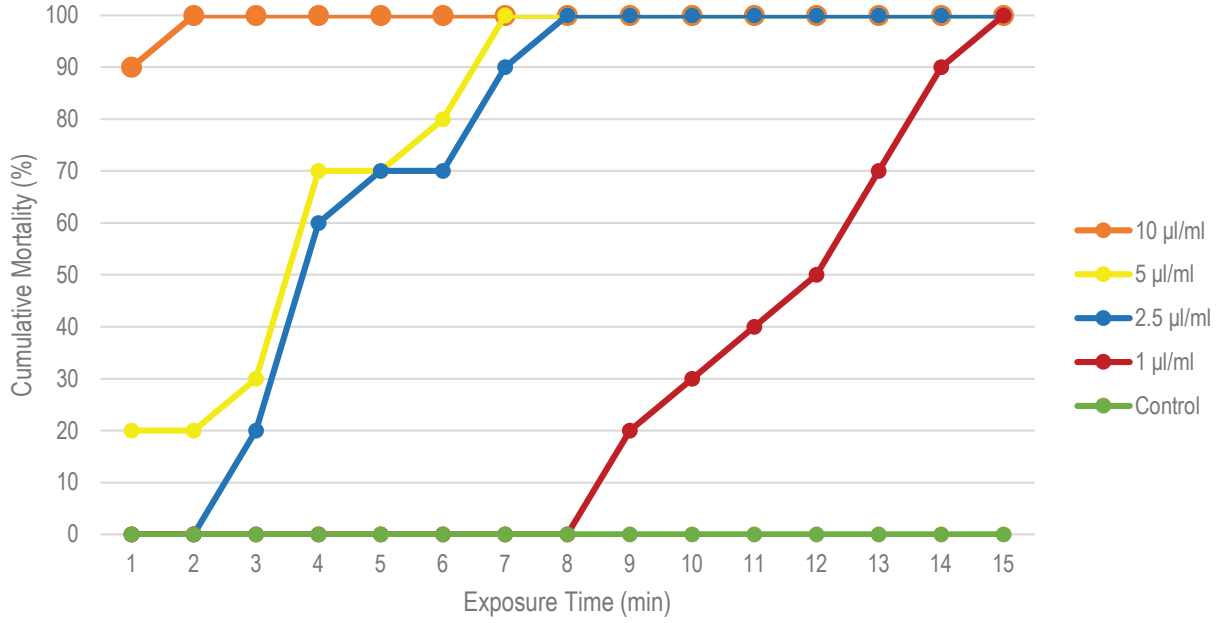

Fig. 1. *In vitro* cumulative mortality of *Dactylogyrus* sp. exposed to peppermint essential oil. Control: 1:50 DMSO.

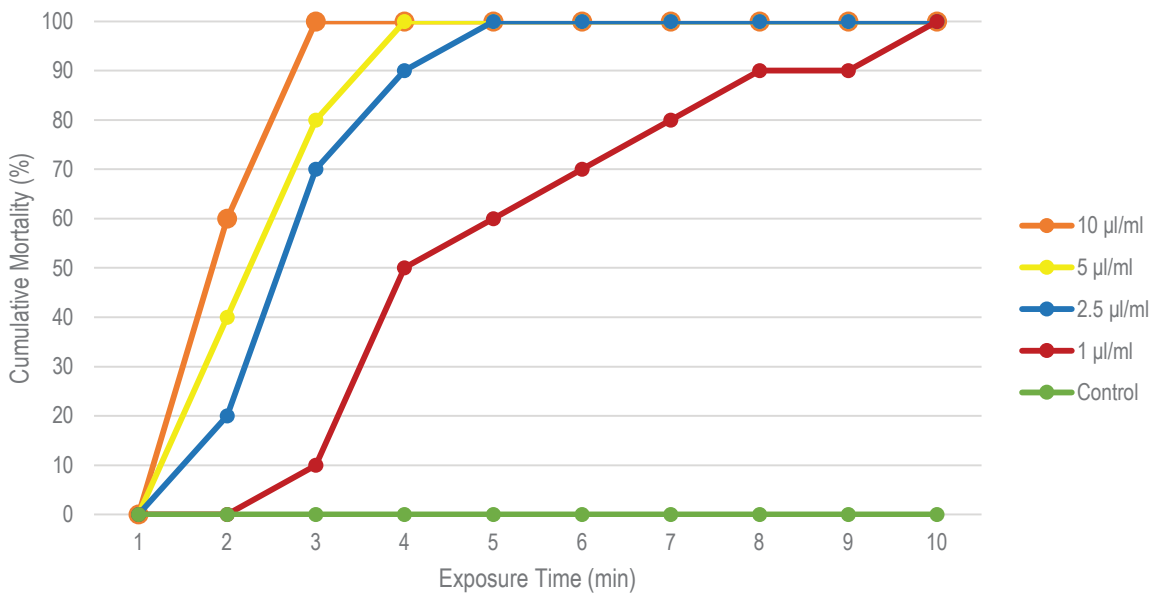

Fig. 2. *In vitro* cumulative mortality of *Dactylogyrus* sp. exposed to lemon essential oil. Control: 1:50 DMSO

In vivo determination of the antiparasitic effect of essential oils on Dactylogyrus sp.

Before performing *in vivo* tests with carp, EC50 concentrations of each herbal essential oil were determined by *in vitro* tests. A total of 40 carp were used in *in vivo* tests. Each essential oil was applied as a single bath to the carp (N=10 for each essential oil application). The control group contained the same number of carp (N=10). EC50 concentrations of peppermint (2.53 µl/ml in 5 min exposure), lemon (0.73 µl/ml in 5 min exposure), and tea tree essential oils (0.30 µl/ml in 2 min exposure) were tested for the respective durations. Treatments were carried out in a 20 L plastic pot containing 5 L of herbal essential oil solutions.

Samples (0.01 g) collected from the gill mucus of each carp before and after bath treatment were placed on glass slides, and non-motile and motile parasites were counted under a light microscope (Nikon 120 model) at 25 Å ~ 10 magnifications.

The antiparasitic efficacy was estimated according to the methods described in the previous study (Zhou *et al*., 2021) and the following formula was used:

Antiparasitic Efficacy = ((A-B) / A) x 100

A: Average number of live parasites in gill mucus before essential oil exposure

B: Average number of live parasites in gill mucus after essential oil exposure

Statistics

Probit analysis was applied to determine EC50 values in *vitro* tests (Finney, 1971) using NCSS 2020 statistics program. In *in vitro tests,* in order to determine the relationship between the mean time to death of parasites and the concentration of the essential oil applied, Pearson's correlation test was used. Statistical analysis for *in vivo* test results was carried out using one-way ANOVA. In all statistical tests, differences in the efficacies of essential oils were considered significant when $p<0.05$.

Ethical Approval and Informed Consent

Fish management and experimental protocols (with the reference number 2020-8-67) approved by the Ankara University Ethics Committee were complied with during the research.

Results

In vitro antiparasitic effect of essential oils on Dactylogyrus sp.

In the *in vitro* tests, the antiparasitic effects of peppermint, lemon, and tea tree essential oils were determined to be time and dose-dependent ($p<0.05$). Using Pearson's correlation test, the relationship between increasing essential oil concentrations and decreasing mean time to nonmotility of parasites was found to be significant ($p<0.05$).

In *in vitro* peppermint essential oil tests, cumulative mortality of *Dactylogyrus* sp. was observed to reach 100 % at 10 µl/ml in 2 min, at 5 µl/ml in 7 min, at 2.5 µl/ml in 8 min and at 1 µl/ml in 15 min (Fig. 1). The antiparasitic effect was observed to increase with increasing concentration and exposure duration.

In *in vitro* lemon essential oil tests, cumulative mortality was 100 % at 10 µl/ml in 3 min, at 5 µl/ml in 4 min, at 2.5 µl/ml in 6 min, and at 1 µl/ml in 10 min. At the lowest concentration (1 µl/ml) using

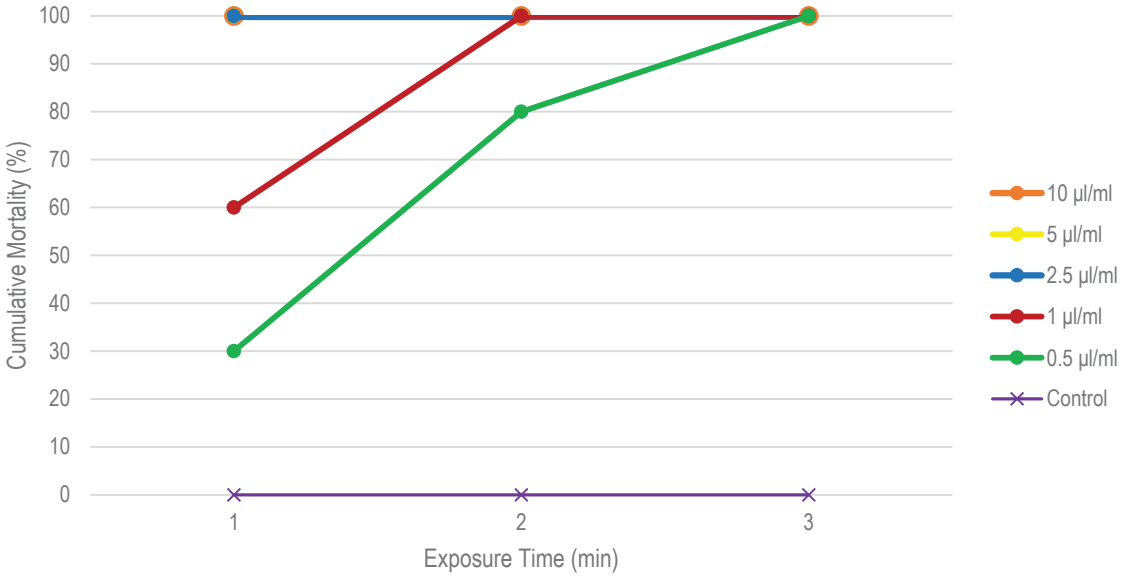

Fig. 3. *In vitro* cumulative mortality of *Dactylogyrus* sp. exposed to tea tree essential oil. Control: 1:50 DMSO

the lemon essential oil, cumulative mortality of *Dactylogyrus* sp. reached 100 % *in vitro* after 10 min (Fig. 2). At the highest concentration (10 µl/ml) all parasites died in 3 min. The antiparasitic activity of lemon essential oil as well increased with concentration and exposure duration.

The antiparasitic effect of tea tree essential oil at 10, 5, 2.5, 1, and 0.5 µl/ml concentrations against *Dactylogyrus* sp. were assessed for exposure durations between 1 and 3 min in *in vitro* tests. In these tests, cumulative mortality was observed to reach 100 % at 10, 5, and 2.5 µl/ml concentrations in 1 min, at 1 µl/ml in 2 min, and at 0.5 µl/ml in 3 min. In the experiments with tea tree essential oil (10, 5, 2.5, 1, and 0.5 µl/ml), cumulative mortality of *Dactylogyrus* sp. reached 100 % in 3 min (Fig. 3). Like peppermint and lemon essential oils, tea tree essential oil demonstrated an increasing antiparasitic effect with increasing concentration and exposure duration.

Dactylogyrus sp. behavior after being exposed in vitro to peppermint, lemon, and tea tree essential oils

In *in vitro* experiments, *Dactylogyrus* sp. was observed to contract rapidly and die after exposure to peppermint, lemon, and tea tree essential oils (Fig. 4).

In vivo antiparasitic effect of essential oils on Dactylogyrus sp.

In *in vivo* tests, EC50 for peppermint (2.53 µl/ml) and lemon (0.73 µl/ml) essential oils in 5 min exposure and EC50 for tea tree (0.30 µl/ml) essential oil in 2 min exposure were applied as a single bath on carp and resulted in a significant reduction in parasite intensity on fish gills ($p<0.05$).

The mean number of *Dactylogyrus* sp. decreased from 50.60 ± 7.78 to 36.30 ± 5.52, from 69.20 ± 21.95 to 47.70 ± 14.71, and from 54.60 ± 7.04 to 35.40 ± 9.58 for peppermint, lemon, and tea tree essential oils, respectively (Fig. 5). The antiparasitic efficacies

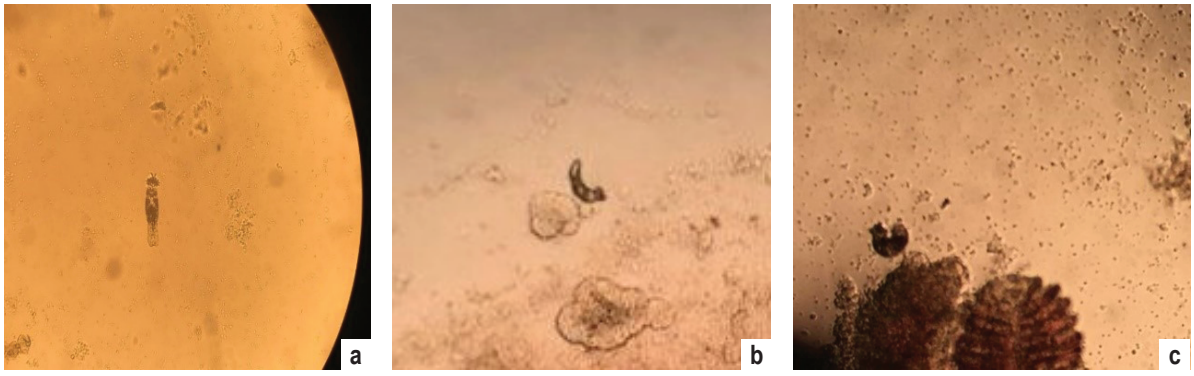

Fig. 4. Parasite behavior; (a) *Dactylogyrus* sp. before exposure (alive); (b) Contraction of *Dactylogyrus* sp. after exposure (c) Death of *Dactylogyrus* sp. after exposure.

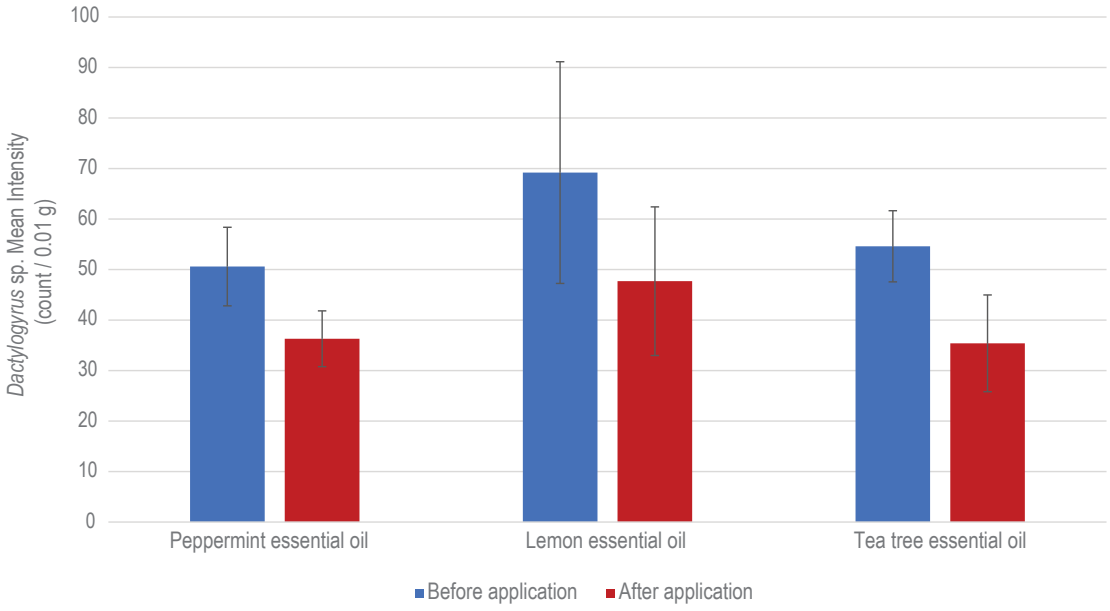

Fig. 5. *Dactylogyrus* sp. mean intensity in carp gills before and after *in vivo* application of peppermint, lemon, and tea tree essential oils.

were calculated as 28.23 % for peppermint essential oil, 30.95 % for lemon essential oil, and 35.31 % for tea tree essential oil.

Discussion

Restriction or prohibition in many countries of traditional chemical therapies, concerning their harmful side effects on fish, humans, and the environment, made it necessary to find alternative therapeutics to combat parasitic diseases in fish. For this reason, there is an increasing amount of research on the potential applications of natural products and herbal substances (Tavares-Dias, 2018). Treatment potentials of various herbal products against different parasites in fish have been studied (Wang *et al*., 2011; Wu *et al*., 2011; Lu *et al*., 2012; Militz *et al*., 2013; Ramudu & Dash, 2013; Valladao *et al*., 2015; Tavares-Dias, 2018; Yavuzcan *et al*., 2019; Yildiz & Bekcan, 2020). However, studies on the antiparasitic effects of essential oils against monogeneans are limited for some fish species. Antiparasitic activity of peppermint, lemon, and tea tree essential oils was assessed in this research *in vitro* and *in vivo* against *Dactylogyrus* sp. on carp.

In the *in vitro* tests of our study, peppermint, lemon, and tea tree essential oils showed different levels of antiparasitic effect against *Dactylogyrus* sp. collected from carp. These effects were determined to be dose and duration dependent and to increase with rising essential oil concentration and exposure time. Similarly, the dose and time-dependent antiparasitic effect of peppermint essential oil were observed in Malheiros *et al*. (2016)'s study, where 80 mg/l, 160 mg/l, and 320 mg/l concentrations of peppermint essential oil were applied *in vitro* on *Dawestrema* spp. While 100 % of parasites were dead in 5 hours at the lowest concentration (80 mg/l), at the highest concentration (320 mg/l) 100 % mortality had been observed in 30 min. In another study, da Costa *et al*. (2017) demonstrated the dose and time-dependent antiparasitic effect of peppermint and tea tree essential oils for monogenean parasites (*Anacanthorus penilabiatus* and *Mymarothecium viatorum*) collected from pacu (*Piaractus mesopotamicus*). *In vitro* antiparasitic effects of peppermint and tea tree essential oils in our study against *Dactylogyrus* sp. are in line with the antiparasitic effects observed in the study of da Costa *et al*. (2017). Time and dose-dependent patterns of antiparasitic effects of peppermint and tea tree essential oil were also observed for Ciliata parasite*, Ichthyophthirius multifiliis* trophonts (Valladão *et al*., 2016). In *in vitro* experiments here, cumulative mortalities of the parasites reached 100 % after treatment with peppermint, lemon, and tea tree essential oils in approximately 15, 10, and 3 minutes, respectively. Thus, the essential oil with the highest anthelmintic effect against *Dactylogyrus* sp. has been tea tree essential oil and the one with the lowest effect has been peppermint essential oil.

Plant sources previously studied for their antiparasitic potential against *Dactylogyrus* sp. were *Ginkgo biloba, Dioscorea zingiberensis* (Jiang *et al*., 2014), ginger bulb (*Zingiber officinale*) and pomegranate peel (*Punica granatum*) (Phan *et al*., 2021). Following ginger bulb and pomegranate peel treatments, the death of *Dactylogyrus* was observed between 1 min to 9 min for the ginger bulb and between 1 min to 15 min for pomegranate peel. The results of Phan *et al*. (2021)'s study are similar to our study in general. Zoral *et al*. (2017) assessed *in vitro* and *in vivo* anthelmintic activity of *Rosmarinus officinalis* against *Dactylogyrus minutus* infections in *C. carpio*. It took 61.8 ± 5.6 min and 7.8 ± 1.4 min to kill 100 % of parasites when exposed to 100 g/L and 200

g/L aqueous rosemary extract solution, respectively. Thus, peppermint, lemon, and tea tree essential oils in our study appear to have higher antiparasitic effects against *Dactylogyrus* sp. than *R. officinalis* against *D. minutus.*

The antiparasitic efficacy of peppermint, lemon, and tea tree essential oils in *in vivo* tests with carp have been found as 28.23 %, 30.95 %, and 35.31 %, respectively. The fact that the antiparasitic effects of the tested essential oils were less in *in vivo* experiments than the antiparasitic effects in *in vitro* experiments, as assessed by EC50, can be due to the ability of parasites to be embedded into skin mucus (Trujillo-González *et al*., 2015; Yildiz & Bekcan, 2020). Hashimoto *et al*. (2016) observed that the monogenean parasite number (*Cichlidogyrus* spp.) in Nile tilapia decreased by 41.63 % after peppermint essential oil at 40 mg/l concentration with recurrent baths. Malheiros *et al*. (2016) investigated the antiparasitic effects of peppermint essential oil, *in vitro* and *in vivo,* against Monogeneans, *Dawestrema cycloancistrium* and *Dawestrema cycloancistrioides,* on *Arapaima gigas* gills. However, the efficacy of peppermint essential oil was low, resulting in no recommendation of its use. Ferreira *et al*. (2019) investigated the antiparasitic effect of peppermint essential oil *in vivo* on *Piscinoodinium pillulare* on *Collosoma macropomum.* The recommended concentration of 20 mg/l applied as short-term baths for 24-hour intervals showed antiparasitic efficacy of 42.97 %. Steverding *et al*. (2005) studied *in vivo* the antiparasitic effects of tea tree essential oil against *Gyrodactylus* spp. in *Gasterosteus aculeatus*, and the number of parasites was found to be reduced by 90 % after 2 days compared to the control group. Baldissera *et al*. (2017) observed that tea tree essential oil used as bath treatment at a concentration of 50 µl/l for 1 hour every day for 4 days on silver catfish (*Rhamdia quelen*) caused a 94.87 % reduction in *I. multifiliis.* The parasite (*D. minutus)* intensities in carp were significantly lower in Zoral *et al*. (2017)'s study when fish were exposed to 50 g/l of aqueous rosemary solution for 30 min. The exposure durations in our *in vivo* experiments were 2 min for tea tree essential oil and 5 min for peppermint and lemon essential oils. Lower values of antiparasitic efficacy of essential oils in our study in comparison to the previous studies can be explained by the short and single treatments with the essential oils tested in the present study.

In vitro and *in vivo* test results of our study presented the antiparasitic potency of lemon essential oil against *Dactylogyrus* sp. in carp. To the best of our knowledge, this is the first study to assess the antiparasitic potential of lemon essential against parasites in fish. The available studies on lemon essential oil were about its capacity to kill bacteria when applied as a feed supplement in different fish species such as *Oreochromis mossambicus* (Baba *et al*., 2016) and *Labeo victorianus (*Ngugi *et al*., 2017).

Some other herbal extracts were also tested against Monogeneans. For example, Ling *et al*. (2015) analyzed the antiparasitic potential of cinnamon essential oil against *Dactylogyrus intermedius* in goldfish under *in vivo* conditions, and anthelmintic efficacies up to 75 % were observed in higher concentrations. Jiang *et al*. (2014) demonstrated the synergistic anthelmintic effect of the combination of *Ginkgo biloba* and *Dioscorea zingiberensis* extracts against *Dactylogyrus* sp. infections in goldfish. Luo *et al*. (2016) studied synergistic combinations of 10 kinds of traditional Chinese medicines against *Dactylogyrus* sp. and found 11 synergistic anthelmintic pairs.

In our study, the bath treatments with peppermint, lemon, and tea tree essential oils resulted in the reduction of *Dactylogyrus* in the gills. The antiparasitic capacities were tea tree essential oil>lemon essential oil> and peppermint essential oil. The differences between the results obtained from *in vitro* experiments and those obtained from *in vivo* experiments can be explained by the fact that the response of biological systems *in vivo* may differ from what is observed *in vitro* due to the complexity of biological interactions such as the effects of the mucus layer in fish. It is important to note that the success of herbal essential oil treatments may not prove as successful in controlling parasites. The effectiveness of the treatment may vary depending on the parasite species, the life cycle of the parasite, and the purity or quality of the essential oil.

Conclusion

In this research, peppermint, lemon, and tea tree essential oils showed antiparasitic effects against *Dactylogyrus* sp. in the gills of carp. In *in vitro* tests, the antiparasitic effects were assessed to be time and dose-dependent. *In vivo* tests conducted on carp demonstrated peppermint, lemon, and tea tree essential oils have relatively reduced antiparasitic effects against *Dactylogyrus* sp. The fact that peppermint, lemon, and tea tree essential oils have the potential to cause a lower impact on *Dactylogyrus* sp. in carp gills.

Conflict of Interest

Authors state no conflict of interest.

References

Baba, E., Acar, U., Ontas, C., Kesbic, O. S., Yilmaz, S. (2016): Evaluation of *Citrus limon* peels essential oil on growth performance, immune response of Mozambique tilapia *Oreochromis mossambicus* challenged with *Edwardsiella tarda*. *Aquaculture*, 465: 13 – 18. DOI: 10.1016/j.aquaculture.2016.08.023

Baldissera, M.D., Souza, C.F., Moreira, K.L., Da Rocha, M.I.U., Da Veiga, M.L., Baldisserotto, B. (2017): *Melaleuca alternifolia* essential oil prevents oxidative stress and ameliorates the antioxidant system in the liver of silver catfish (*Rhamdia quelen*) naturally infected with *Ichthyophthirius multifiliis*. *Aquaculture*, 480: 11 – 16. DOI: 10.1016/j.aquaculture.2017.07.042

Bruno, D.W., Nowak, B., Elliott, D.G. (2006): Guide to the identification of fish protozoan and metazoan parasites in stained tissue sections. *Dis Aquat Organ*, 70: 1 – 36. DOI: 10.3354/dao070001

Bulfon, C., Volpatti, D., Galeotti, M. (2015): Current research on

the use of plant-derived products in farmed fish. *Aquac Res*, 46(3): 513 – 551. DOI: https://doi.org/10.1111/are.12238s

Da Costa, J.C., Valladão, G.M.R., Pala, G., Gallani, S.U., Kotzent, S., Crotti, A.E.M., Fracarolli, L., Da Silva, J.J.M. Pilarski, F. (2017): *Copaifera duckei* oleoresin as a novel alternative for treatment of monogenean infections in pacu *Piaractus mesopotamicus*. *Aquaculture*, 471: 72 – 79. DOI: 10.1016/j.aquaculture.2016.11.041

Ernst, I., Whittington, I., Corneillie, S., Talbot, C. (2002): Monogenean parasites in sea-cage aquaculture. *Austasia Aquacult*, 16: 46 – 48

FAO (2022): *The State of World Fisheries and Aquaculture 2022*. Towards Blue Transformation. Rome. DOI: 10.4060/cc0461en

Ferreira, L.C., Da Cruz, M.G., Lima, T.B.C., Serra, B.N.V., Chaves, F.C.M., Chagas, E.C., Ventura, A.S., Jerônimo, G.T. (2019): Antiparasitic activity of *Mentha piperita* (Lamiaceae) essential oil against *Piscinoodinium pillulare* and its physiological effects on *Colossoma macropomum* (Cuvier, 1818). *Aquaculture*, 512: 734343. DOI: 10.1016/j.aquaculture.2019.734343

Finney, D.J. (1971): *Probit Analysis. 3rd Edition*, Cambridge University Press, 333 pp.

Freire, M.M., Jham, G.N., Dhingra, O.D., Jardim, C.M., Barcelos, R.C., Valente, V.M.M. (2012): Composition, antifungal activity and main fungitoxic components of the essential oil of *Mentha piperita* L. *J Food Saf*, 32(1): 29 – 36. DOI: 10.1111/j.1745-4565.2011.00341.x

Hashimoto, G.S.O., Marinho Neto, F., Ruiz, M.L., Acchile, M., Chagas, E.C., Chaves, F.C.M., Martins, M.L. (2016): Essential oils of *Lippia sidoides* and *Mentha piperita* against monogenean parasites and their influence on the hematology of Nile tilapia. *Aquaculture,* 450: 182 – 186. DOI: 10.1016/j.aquaculture.2015.07.029

Jiang, C., Wu, Z.Q., Liu, L., Liu, G.L., Wang, G.X. (2014): Synergy of herbal ingredients combination against *Dactylogyrus* spp. in an infected goldfish model for monogenean management. *Aquaculture*, 433:115 – 118. DOI: 10.1016/j.aquaculture.2014.05.045

Kumar, K.V., Patra, D.D. (2012): Alteration in yield and chemical composition of essential oil of *Mentha piperita* L. plant: effect of fly ash amendments and organic wastes. *Ecol Eng*, 47: 237 – 241. DOI: 10.1016/j.ecoleng.2012.06.019

Lieke, T., Meinelt, T., Hoseinifar, S.H., Pan, B., Straus, D.L., Steinberg, C.E.W. (2020): Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. *Rev Aquac*, 12: 943 – 965. DOI: 10.1111/raq.12365

Ling, F., Jiang, C., Liu, G., Li, M., Wang, G. (2015): Anthelmintic efficacy of cinnamaldehyde and cinnamic acid from cortex cinnamon essential oil against *Dactylogyrus intermedius*. *Parasitology,* 142: 1744 – 1750. DOI: 10.1017/S0031182015001031

Lom, J., Dyková, I. (1992): *Protozoan parasites of fishes. Developments in Aquaculture and Fisheries Science*, 26. Elsevier Sci. Publ. B.V., Amsterdam. pp. 315

Lu, C., Zhang, H.Y., Ji, J., Wang, G.X. (2012): *In vivo* anthelmintic activity of *Dryopteris crassirhizoma, Kochia scoparia*, and *Polygala tenuifolia* against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*). *Parasitol Res*, 110(3): 1085 – 1090. DOI: 10.1007/s00436-011-2592-0

Luo, F., Ling, F., Geng, T., Liu, G., Jiang, C., Wang, G. (2016): Systematic screening identifies synergistic combinations of traditional Chinese medicines and ingredients against *Dactylogyrus* infections using a goldfish model. *Aquaculture*, 459: 198 – 202. DOI: 10.1016/j.aquaculture.2016.03.045

Malheiros, D.F., Maciel, P.O., Videira, M.N., Tavares-Dias, M. (2016): Toxicity of the essential oil of *Mentha piperita* in *Arapaima gigas* (pirarucu) and antiparasitic effects on *Dawestrema* spp. (Monogenea). *Aquaculture,* 455: 81 – 86. DOI: 10.1016/j.aquaculture.2016.01.018

Malmberg, G. (1970): The excretory system and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). *Ark. Zool.*, 23: 235

Militz, T.A., Southgate, P.C., Carton, A.G., Hutson, K.S. (2013): Dietary supplementation of garlic (*Allium sativum*) to prevent monogenean infection in aquaculture. *Aquaculture*, 408: 95 – 99. DOI: 10.1016/j.aquaculture.2013.05.027

Ngugi, C.C., Oyoo-Okoth, E., Muchiri, M. (2017): Effects of dietary levels of essential oil (EO) extract from bitter lemon (*Citrus limon*) fruit peels on growth, biochemical, haemato-immunological parameters and disease resistance in Juvenile *Labeo victorianus* fingerlings challenged with *Aeromonas hydrophila*. *Aquac Res*, 48(5): 2253 – 2265. DOI: 10.1111/are.13062

Obiekezie, A.I., Taege, M. (1991): Mortalities in hatchery-reared fry of the African catfish, *Clarias gariepinus* (Burchell) caused by *Gyrodactylus groschafti*, Ergens, 1973. *Bull Eur Ass Fish Pathol,* 11: 82 – 85

Phan, V.Q., Harmansa, Y.B., Yavuzcan, H. (2021): *In vitro* Antiparasitic Activity of Ginger (*Zingiber officinale*) Bulb and Pomegranate (*Punica granatum*) Peel Against Monogenean Fish Parasite., *Dactylogyrus* sp. *Acta Aquat. Turc.*, 17 (1): 56 – 63. DOI: 10.22392/actaquatr.751913

Ramudu, K.R, Dash, G. (2013): A review on herbal drugs against harmful pathogens in aquaculture. *Am. J. Drug Discov. Dev.*, 3(4): 209 – 219. DOI: 10.3923/ajdd.2013.209.219.

Reed, P., Francis-Floyd, R., Klinger, R., Petty, D. (2009): *Monogenean parasites of fish. Fisheries and aquatic sciences*. University of Florida UF, IFAS Extension. FA28, USA, 4: 1 – 4

Shinn, A., Pratoomyot, J., Bron, J., Paladini, G., Brooker, E., Brooker, A. (2015): Economic impacts of aquatic parasites on global finfish production. *Glob Aquac Advocate*: 82 – 84

Soares, B.V., Tavares-Dias, M. (2013): Espécies de Lippia (Verbenaceae), seu potencial bioativo e importância na medicina veterinária e aquicultura. [Lippia species (Verbenaceae), their bioactive potential and importance in veterinary medicine and aquaculture] *Biota Amaz,* 3: 109 – 123 (In Portuguese)

Soares, B.V., Neves, L.R., Oliveira, M.S.B., Chaves, F.C.M., Dias, M.K.R., Chagas, E.C., Tavares-Dias, M. (2016): Antiparasitic activity of the essential oil of *Lippia alba* on ectoparasites of *Colossoma*

macropomum (tambaqui) and its physiological and histopathological effects. *Aquaculture,* 452: 107 – 114. DOI: 10.1016/j.aquaculture.2015.10.029

Soares, B.V., Neves, L.R., Ferreira, D.O., Oliveira, M.S.B., Chaves, F.C.M., Chagas, E.C., Gonçalves, R.A., Tavares-Dias, M. (2017a): Antiparasitic activity, histopathology and physiology of *Colossoma macropomum* (tambaqui) exposed to the essential oil of *Lippia sidoides* (Verbenaceae). *Vet Parasitol,* 234: 49 – 56. DOI: 10.1016/j.vetpar.2016.12.012

Soares, B.V., Cardoso, A.C.F., Campos, R.R., Gonçalves, B.B., Santos, G.G., Chaves, F.C.M., Chagas, E.C., Tavares-Dias, M. (2017b): Antiparasitic, physiological and histological effects of the essential oil of *Lippia origanoides* (Verbenaceae) in native freshwater fish *Colossoma macropomum*. *Aquaculture,* 469: 72 – 78. DOI: 10.1016/j.aquaculture.2016.12.001

Steverding, D., Morgan, E., Tkaczynski, P., Walder, F., Tinsley, R. (2005): Effect of Australian tea tree oil on *Gyrodactylus* spp. infection of the three-spined stickleback *Gasterosteus aculeatus*. *Dis Aquat Organ*, 66(1): 29 – 32.

Tavares-Dias, M. (2018): Current knowledge on use of essential oils as alternative treatment against fish parasites. *Aquat Living Resour*, 31: 13. DOI: 10.1051/alr/2018001

Thomsen, P.S., Jensen, T.M., Hammer, K.A., Carson, C.F., Mølgaard, P., Riley, T.V. (2011): Survey of the antimicrobial activity of commercially available Australian tea tree (*Melaleuca alternifolia*) essential soleroil products *in vitro*. *J Altern Complement Med*, 17: 835 – 841. DOI: 10.1089/acm.2010.0508

Trujillo-González, A., Constantinoiu, C.C., Rowe R., Hutson, K.S. (2015): Tracking transparent monogenean parasites on fish from infection to sexual maturity. *Int J Parasitol Parasites Wildl*, 4: 316 – 322. DOI: 10.1016/j.ijppaw.2015.06.002

Tsai, M.L., Wu, C.T., Lin, T.F., Lin, W.C., Huang, Y.C., Yang, C.H. (2013): Chemical composition and biological properties of essential oils of two mint species. *Trop J Pharm Res*, 12(4): 577 – 582. DOI: 10.4314/tjpr.v12i4.20

Valladão, G.M.R., Gallani, S.U., Pilarski, F. (2015): Phytotherapy as an alternative for treating fish disease. *J Vet Pharmacol Ther*, 38(5): 417 – 428. DOI: 10.1111/jvp.12202

Valladão, G.M.R., Gallani, S.U., Ikefuti, C.V., Da Cruz N Levy-Pereira, C., Rodrigues, M.V.N., Pilarski, F. (2016): Essential oils to control *ichthyophthiriasis* in pacu, *Piaractus mesopotamicus* (Holmberg): special emphasis on treatment with *Melaleuca alternifolia*. *J Fish Dis,* 39(10): 1143 – 1152. DOI: 10.1111/jfd.12447

Wang, Y., Wu, Z.F., Wang, G.X., Wang, F., Liu, Y.T., Li, F.Y., Han, J. (2011): *In vivo* anthelmintic activity of bruceine A and bruceine D from *Brucea javanica* against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*). *Veterinary Parasitol,* 177(1–2): 127 – 133. DOI: 10.1016/j.vetpar.2010.11.040

Wu, Z.F., Zhu, B., Wang, Y., Lu, C., Wang, G.X. (2011): *In vivo* evaluation of anthelmintic potential of medicinal plant extracts against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*). *Parasitol Res*, 108(6): 1557 – 1563. DOI: 10.1007/s00436-010-2211-5

Yao J.Y., Zhou Z.M., Pan X.Y., Hao G.J., Li X.L., Xu Y., Shen J.Y., Ru H.S., Yin W.L. (2011): In *vivo* anthelmintic activity of chelidonine from *Chelidonium majus L*. against *Dactylogyrus intermedius* in *Carassius auratus*. *Parasitol Res,* 109: 1465 – 1469. DOI: 10.1007/s00436-011-2416-2

Yavuzcan, Y.H., Phan, V.Q., Parisi, G., Dam S.M. (2019): Anti-parasitic activity of garlic (*Allium sativum*) and onion (*Allium cepa*) juice against crustacean parasite, *Lernantropus kroyeri*, found on European sea bass (*Dicentrarchus labrax*). *Ital J Anim Sci*, 18(1): 833 – 837. DOI: 10.1080/1828051X.2019.1593058

Yildiz Y.H., Bekcan, S. (2020): Control of ectoparasitosis in carp (*Cyprinus carpio*) induced by *Gyrodactylus elegans* (Monogenea) with garlic (*Allium sativum*) and onion (*Allium cepa*) extracts. *Ecocycles*, 6(1): 10 – 17. DOI: 10.19040/ecocycles.v6i1.157

Zhou, S., Dong, J., Liu, Y., Yang, Q., Xu, N., Yang, Y., Ai, X. (2021): Antiparasitic Efficacy of Herbal Extracts and Active Compound Against *Gyrodactylus kobayashii* in *Carassius auratus*. *Front Vet Sci*, 8: 665072. DOI: 10.3389/fvets.2021.665072

Zoral, M.A., Futami, K., Endo, M., Maita, M., Katagiri, T. (2017): Anthelmintic activity of *Rosmarinus officinalis* against *Dactylogyrus minutus* (Monogenea) infections in *Cyprinus carpio*. *Vet Parasitol*, 247: 1 – 6. DOI: 10.1016/j.vetpar.2017.09.013