Use of lavender and wild thyme to make aquatic filters in fish farming

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Abstract. Maintaining the medial parameters of water quality and immune stimulation of the *Heteroclarias* hybrid under conditions of intensive growth is a constant interest of growth technologies. For this purpose, we used filters consisting of lavender, thyme and a mixture of the two plants, to observe their influence on the hybrid's growth performance. Four groups, three experimental (LL), (LT), (LLT) and one control (LM), consisting of 24 juvenile *Heteroclarias* individuals each, were observed for 16 weeks. Water quality parameters and fish growth were monitored, and blood parameters were analyzed. Exposure to plant inclusion in filters did not affect the monitored parameters.
Key Words: *Heteroclarias*, medial water parameters, plant filters, productive effect.

Introduction. Medicinal and aromatic plants, available as such or in the form of extracts, have shown their effectiveness in preventing and ameliorating some diseases in both humans and animals (Bulfon et al 2013; Mateescu et al 2014; Tadese et al 2021).

In order to meet the high demand for aquaculture products and to reduce the pressure on the natural environment, intensive aquaculture systems and technologies are widely used in the world today (FAO 2018, 2020; Jana et al 2018; Oddsson 2020; Taştan & Salem 2021).

Ensuring the welfare and prevention of disease in fish and aquatic organisms, as part of aquaculture intensification, has attracted increased interest with the use of medicinal and aromatic plants (Van Hai 2015; Tavares-Dias 2018; Gabriel 2019; Anastasiou et al 2020; Hernández-Contreras & Hernández 2020; Gupta et al 2021; Faheem et al 2022). Plant parts, whole plants or extracts were introduced into the growing water (Caruso et al 2013; Caruso et al 2017; Raman 2017; Reverter et al 2017; Tadese et al 2021) and they have also been used as immunostimulants administered orally. Less often, they have been applied by injection or immersion for preventive purposes or as treatment (Harikrishnan et al 2011; Bulfon et al 2013).

Different species of lavender (genus *Lavandula*) and thyme (genus *Thymus*) from the *Lamiaceae* family have properties that recommend them for medicinal use, in the food and pharmaceutical industries (Samarth et al 2017; Nieto 2017, 2020; da Silva et al 2021). The active biological compounds represented by linalool, 1,8 cineole, borneol, camphor, linalyl acetate, lavandulyl acetate, thymol and carvacrol (Varga et al 2015; Zeljković & Maksimović 2015; Aprotosoaie et al 2017; Wels et al 2018) give these plants antioxidant, antimicrobial, anti-inflammatory and immunostimulating properties (Cardia et al 2018; Capatina et al 2021; Boaru et al 2022).

On fish, lavender (*Lavandula angustifolia* Mill.) in the form of oil or various extracts has been proven to have sedative and slightly anesthetic, antimicrobial, anti-inflammatory effects and also a positive effect on the growth rate (Wimalasena et al 2018; Hajhashemi et al 2003; Aydin & Barbas 2020).

In aquaculture, wild thyme *Thymus serpyllum* L. whose chemical composition gives it medicinal, culinary, healing and phytotherapeutic properties (Mašković et al 2017; Li et al 2019) has been tested for its antioxidant and antibacterial effects

(Wunderlich et al 2017; Honcharenko et al 2018). In the same idea, plants from the *Thymus* genus have proven their effectiveness in preventing and treating some diseases, but also in increasing immunity and improving the production performance of some fish species (Yassen et al 2017; Hoseini & Yousefi 2018; Nieto 2020).

Analyzing the different benefits and applications of medicinal and aromatic plants, the aim of this work was to use lavender and thyme in making natural water filters. Whether these plants used alone or in combination influence water quality, growth and health of fish was investigated.

Material and Method

Experiment preparation and obtaining natural aquatic filters. The experiment was carried out in 2021 in the Aquaculture laboratory, Faculty of Animal Science and Biotechnology, USAMV Cluj-Napoca.

Lavender (*L. angustifolia*) and wild thyme (*T. serpyllum*) came from an organic crop in Sălaj county. From each species, whole plants were harvested and dried in a shaded and well-ventilated space. After drying, they were crushed and 6 g of each product was placed in bags made of linen. We thus obtained three types of natural aquatic biofilters, hereinafter L for lavender, T for wild thyme, LT for lavender and wild thyme mixture.

The establishment of fish groups according to the aquatic filter used. The biological material was comprised of 21-day-old hybrid African catfish (*Heterolarias*) and was purchased from a fish farm in Arad County, Romania. The fish were transported from the farm to the laboratory in containers, at a water temperature of 26°C. In the laboratory, the fish were transferred to an acclimatization tank (100 L), in which the water temperature was maintained in the range 26-28°C. After the acclimatization period (14 days), four lots were formed with 24 specimens per lot. For this purpose, glass aquariums (40x25x27 cm, water volume 25 L) and SERA FIL 60/120 filter pumps were used. The control lot (LM) was equipped with the original filter pump. In the case of the experimental lots, the sponge in the filter cartridge of the pump was replaced with the natural biofilters manufactured. This resulted in the lavender batch (LL), the thyme batch (LT) and the lavender and thyme mixture batch (LLT).

Monitoring of the physicochemical parameters of water and aquarium maintenance. Throughout the experiment, stored tap water was used. Water parameter data were collected daily for temperature and pH, while dissolved oxygen (DO), ammonium and nitrite were determined weekly, with an individual thermometer for each aquarium, digital pH-meter (resolution of 0.1 units) and a colorimetric kit (JBLProAqu Test Combi Set Plus).

The filter pumps were cleaned every two days in the portion of the terminal cartridge, and once a week they were fully washed and cleaned, at which stage each natural biofilter was replaced with a new one. Daily, 20% of the water in the aquariums was replaced in the first month, 50% in the second month and 80% in the third month. A hand pump was used to carry out the water changes and the solids were gently cleaned from the bottom of the aquariums.

Fish feeding and evolution. Both during the acclimatization and the experimental period, the fish were fed with commercial feed (Alltech Coppens), granulation 0.8 mm (56% protein), 1.5 mm (54% protein) and 3 mm (45% protein). The amount of feed administered represented 3% of the total biomass, daily. The fish in each batch were individually weighed every two weeks and the feeding plan was adjusted according to the total biomass. Feeding was done between 8 am and 4 pm and 4 meals per day were administered. Before each weighing and at the end of the experiment, the feeding of the fish was stopped for 24 hours. In accordance with the measurements carried out, the experimental period was divided into seven growth stages, further identified with I, II, III, IV, V, VI, VII. For each growth stages, the mortalities of each lot were recorded.

Blood biochemical parameters. At the end of the experimental period, blood was collected from two specimens from each group, the fish being anesthetized beforehand with pure clove oil. The time required for anesthesia and recovery after anesthesia and blood collection was timed for each individual specimen. The caudal vein puncture procedure was followed according to the protocol specified for *Clarias batrachus* by Argungu et al (2017). Blood collection was done with a sterile syringe and each sample (2 mL) was placed in 4 mL vacutainers with heparin. The samples were processed on the same day in the hematology laboratory of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania. The Screen Master Touch UV-VIS analyzer with a commercial kit was used. Spectrophotometric techniques were used for the tracked profiles with readings of the wavelength (λ) specific to each tracked parameter.

Statistical data processing. The raw data obtained by weighing the biological material, as well as those regarding the physicochemical parameters of the water were statistically processed (GraphPad Prism 8 program). Differences between means were analyzed by the Tukey multiple comparisons test ($p<0.05$).

Results and Discussion

Water quality. The average values regarding the water quality parameters of each batch, for the entire experimental period, are presented in Table 1.

During the entire experimental period, it was ensured that the water temperature was the same in all groups. The table shows the stability of this parameter at 27°C, without any differences between the four batches. The average values recorded by us are within the limits mentioned by other authors for the growth of the juvenile African catfish and *Heteroclarias* (Adewumi et al 2014; Ofonime & Gift 2017, 2019).

Table 1

Water quality parameters depending on the water filter used

Parameter	*Lot*			
	LM	*LL*	*LT*	*LLT*
Temperature T (°C)	27.30±0.12^a	27.20±0.14^a	27.20±0.17^a	27.00±0.18^a
pH	6.60±0.06^a	6.71±0.06^a	6.74±0.05^a	6.70±0.05^a
Dissolved oxygen (mg L^{-1})	3.50±0.52^a	4.00±0.45^b	3.10±0.23^a	3.10±0.21^a
Amonium NH_4 (mg L^{-1})	2.87±0.63^a	2.87±0.58^a	3.18±0.62^a	3.26±0.63^a
Nitrites NO_2 (mg L^{-1})	0.04±0.01^a	0.03±0.00^a	0.03±0.01^a	0.09±0.03^a

Note: values represent mean ± standard error; different superscripts show significant differences ($p<0.05$); LM - control; LL - lavender lot; Lt - thyme lot; LLT - lavender and thyme lot.

Due to the physicochemical and biological processes taking place in water, pH can change rapidly and is a very important parameter in terms of water quality. In the growth of African catfish and *Heteroclarias*, a pH of water between 6.5 and 8 is reported in various studies (Adebayo 2017; Marimuthu et al 2019; Agboola et al 2020). The average values recorded by us were below 7 and express the slightly acidic character (6.69) of the water. There were no differences between the groups, which shows that the natural biofilters used did not influence the water pH in any of the experimental groups.

For DO, there were no differences between the experimental groups LT, LLT and the control group LM, the average values being 3.1 mg L^{-1} and 3.5 mg L^{-1}, respectively. In the case of the experimental group LL, a slight increase in the amount of oxygen in the water was found to 4 mg L^{-1}, a value that indicates differences compared to the other groups. However, the requirements for DO in the water are reduced for African catfish and *Heteroclarias* and in intensive growth values between 3-6 mg L^{-1} represent an optimal range (Ofonime et al 2018; Păpuc et al 2019). In the case of the batch where we used the lavender biofilter, a higher value of DO was recorded, most likely due to the reduction in density as an effect of losses, an aspect that is also found in the total biomass of fish.

Nitrogenous substances (ammonium and nitrites) present in the water in all four aquariums were within the limits allowed in fish growth (Lujerdean 2006; Munteanu & Bogatu 2003). The average value recorded for ammonium was in the range of 2.87-3.26 mg L^{-1}, tolerable and in agreement with the levels accepted in African catfish breeding (Bovendeur et al 1987; Păpuc et al 2019). For nitrites, very close average values are noted (0.03, 0.04 and 0.09 mg L^{-1}) and which also fall within the tolerance limit (Păpuc et al 2019). Ammonia resulting from protein metabolism or from decaying organic matter can be removed by water changes or can be decomposed with the help of a biofilter. In our experiment, this is represented by the absorbent sponge for the control batch and the plant-based product/medicinal product used in the case of experimental batches. The average values obtained for both ammonium and nitrite were very close and, by comparison, no differences were recorded according to the batch of fish and the aquatic filter used. This indicates that there were no accumulations involving some degree of toxicity and affecting the health of the fish.

Evolution of biological material. The average values and the expression of the differences between them in terms of body weight by growth stages are presented in Table 2. The analysis of the data obtained by the initial weighing of the fish from each batch shows that there were no differences between the batches.

Table 2

The growth dynamics of *Heteroclarias* fry depending on the lots

Lot	*Growth stages*							
	Initial	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*
LM	n=24 5.93±0.26[a]	n=24 8.92±0.52[a]	n=23 12.07±0.75[a]	n=21 20.06±0.92[a]	n=21 28.12±1.81[a]	n=21 42.84±3.84[a]	n=21 61.53±6.41[a]	n=21 75.85±8.84[a]
LL	n=24 6.06±0.27[a]	n=23 8.48±0.48[a]	n=22 12.12±0.75[a]	n=18 21.43±1.22[a]	n=18 29.26±2.06[a]	n=17 44.13±3.52[a]	n=17 58.03±3.88[a]	n=17 69.22±4.39[a]
LT	n=24 5.25±0.32[a]	n=23 7.13±0.52[a]	n=22 11.75±0.84[a]	n=21 19.60±1.22[a]	n=19 29.98±2.07[a]	n=19 44.23±3.52[a]	n=19 62.79±5.63[a]	n=19 79.13±7.82[a]
LLT	n=24 6.14±0.29[a]	n=24 8.58±0.52[a]	n=23 13.16±0.78[a]	n=22 21.35±1.05[a]	n=22 30.05±1.40[a]	n=22 45.49±3.15[a]	n=22 62.52±5.18[a]	n=22 78.51±7.27[a]

Note: n - number of specimens; values for body weight express mean ± standard error; different superscripts show significant differences ($p<0.05$); LM - control; LL - lavender lot; Lt - thyme lot; LLT - lavender and thyme lot.

Regarding the evolution of fish in all lots, it falls within the reference limits for intensive growth in *Heteroclarias* fry (Adebayo 2017; Ayanwale et al 2020). During the entire experimental period, losses were recorded in each batch, expressed as a percentage: 12.5% (LM), 29.17% (LL), 20.83% (LT) and 8.33% (LLT). Losses due to cannibalism, a behavior specific to African catfish and its hybrids (Maradun et al 2019) are within the limits also reported by other authors (Abu et al 2010; Agboola et al 2020).

There are some differences between the lots and depending on the growth stages. From the 24 specimens in each batch, the least losses were recorded for LM and LLT, (3 and 2, respectively) and which manifested themselves up to growth stages. On the other hand, 7 specimens from the LL batch and 5 specimens from the LT batch were lost and the survival of the biological material from these batches was stable starting from growth stage V.

Considering the water quality parameters and the losses recorded in each batch, fish growth was homogeneous. Based on the data obtained at each weighing, average values of body weight were very close and without differences between groups. At the end, even if statistically no differences were recorded, it can be seen that, compared to the number of fish that was smaller in the LL group, the average weight obtained was 69.22 g. In contrast, the LLT lot achieves an average weight of 78.51 g. Also, in the case of the control lot LM, the 21 specimens achieve an average weight of 75.85 g and the lot LT obtains an average of 79.13 g reported to 19 specimens. This suggests that fish density is not an influencing factor in this case.

Blood parameters analysis. The transport in the body of various substances dissolved in water is carried out by the blood. Hematology can provide some clues regarding the health and welfare of fish (Bulfon et al 2013; Bake et al 2019; Ofonime & Gift 2019; Tiamiyu et al 2019). Depending on the profile analyzed and differentiated by lots, the results of the blood parameters are presented in Table 3.

Table 3

Biochemical blood parameters in *Heteroclarias* juvenile

Parameter	*Lot*			
	LM	*LL*	*LT*	*LLT*
Hb (g dL^{-1})	8.50	6.28	9.24	8.17
Hct (%)	29.00	19.50	30.00	31.50
Ery (mL mm^{-3})	3.24	2.58	3.36	3.40
Prot (g dL^{-1})	3.55	2.96	3.75	3.73
Alb (g dL^{-1})	1.09	0.97	1.12	1.19
Cholest (mg dL^{-1})	125.30	104.10	167.35	133.20
Tgl (mg dL^{-1})	228.10	174.15	357.40	265.40

Note: Hb - hemoglobin; Hct - hematocrit; Ery - red blood cells; Prot - proteins; Alb - albumins; Cholest - cholesterol; Tgl - triglycerides.

The hemoglobin concentration varied between 6.28-9.24 g dL^{-1}. The studies carried out on different species of fish indicate for this parameter values between 2.6-19.9 g dL^{-1} (Esmaeili 2021). In African and hybrid catfish, reference values (4.70-10.85 g dL^{-1}) have been shown to differ depending on sex, age, weight or diet administered (Gabriel et al 2004; Fagbenro et al 2013; Bake et al 2019).

Hematocrit is a factor that correlates with increased production in animals (Esmaeili 2021) and for catfish and hybrids they are within limits of 20-37% (Argungu et al 2021). The results obtained by us show the range of 29.00-31.50% for the LM, LT and LL lots and a lower percentage of 19.50% in the LL lavender lot. This lower hematocrit value may suggest that the fish were exposed to a stress factor. The argumentation of the observation is supported by the fact that hemoglobin also recorded the lowest value in the fish from the LL group. The decrease in the values of these parameters, as Argungu et al (2021) and Esmaeili (2021) suggest, can be correlated with the effect of a stress factor.

An important indicator of environmental changes is the number of blood cells. The low number of erythrocytes can signal the presence or the start of some processes of degradation of the body. In the case of the LL group, the average number of erythrocytes (2.58) was lower compared to the other groups where 3.24 (LM), 3.36 (LT) and 3.40 mL mm^{-3} (LLT), but these values fall within the reference range for African catfish and hybrid catfish (Adeyemo 2007; Fagbenro et al 2013; Bake et al 2019).

Total serum protein is a commonly measured blood parameter that reveals the close interaction with the immune system. In fish, Esmaeili (2021) reports an average value of 3.60 g dL^{-1} for total blood proteins. In juvenile African catfish, Adeshina et al (2016) indicate an average of 5.47 g dL^{-1} for total protein and 1.53 g dL^{-1} for albumin. Within the protein profile determined by us in *Heteroclarias* juvenile it is observed that the fish from the LT and LLT groups had very close values for total proteins (3.75 and 3.73 g dL^{-1}), followed by the LM group (3.55 g dL^{-1}) and the LL group (2.96 g dL^{-1}). In the case of albumins, the lower value recorded in fish from the LL group (0.97 g dL^{-1}) is also observed, followed by LM (1.09 g dL^{-1}), LT (1.12 g dL^{-1}) and LLT (1.19 g dL^{-1}). The different values recorded in the LT and LLT groups for both total proteins and albumins may suggest a positive immune response resulting from the biologically active compounds of lavender and wild thyme, or their cumulative effect. The observation is also supported by Bulfon et al (2013) who suggest that different biologically active plant compounds increase total serum proteins, albumin and globulin, resulting in stimulation of the humoral immune response and improvement of health status. On the other hand, the slight decrease of these values in the case of the LL group may suggest some secondary reactions, considering the fact that, as Mazandarani et al (2017) also show, fish exposed to linalool can also express an increased level of cortisol.

Within the lipid profile, cholesterol and triglycerides were determined. Compared to the LM control group, the mean values for these parameters were lower in the LL group and higher for the LT and LLT groups. For cholesterol, the fish in the LL group had an average of 104.10 mg dL^{-1}, after which, in ascending order, there were the LM group with 125.30 mg dL^{-1}, LLT with 133.20 mg dL^{-1} and LT with 167.35 mg dL^{-1}. In the case of triglycerides, the order was the same, with the lowest value for group LL (174.15 mg dL^{-1}), followed by groups LM (228.10 mg dL^{-1}), LLT (265.40 mg dL^{-1}) and LT (357.40 mg dL^{-1}). In African catfish, it has been shown that cholesterol levels are influenced by stocking density and fish weight (Okorie-Kanu & Unakalamba 2014). On the other hand, anesthetic substances can change blood indices. In an experiment carried out on young African catfish in which different doses of clove oil were used, Adeshina et al (2016) determined cholesterol values in the range of 161.95-243.83 mg dL^{-1}. The higher values recorded in the lipid profile in the LLT and LT groups may be due to the presence of the thyme, which possibly influenced the appetite and assimilation to a greater extent of the fish food. In the same idea, various studies indicate that wild thyme acts on the increase of blood biochemical parameters and production performances (Yassen et al 2017; Nieto 2020).

Anesthesia and recovery of the fish proceeded under optimal conditions and no mortalities were recorded (Table 4). Depending on the group, the average time required to anesthetize the fish was recorded as follows: LLT (5.57 min), LT (5.90 min), LM (6.03 min) and LL (6.18 min). The time required for the recovery of the fish from anesthesia was in the following order: LM (13.75 min), LT (13.94 min), LLT (14.84 min) and LL (15.19 min).

Table 4

Monitoring of the fish

Lot	*Anesthesia time (minutes)*		*Recovery time (minutes)*	
	Specimen 1	*Specimen 2*	*Specimen 1*	*Specimen 2*
LM	5.81	6.25	13.46	14.05
LL	6.15	6.22	16.11	14.28
LT	5.93	5.88	14.02	13.87
LLT	5.45	5.70	14.51	15.18

The response of different fish species to exposure to different anesthetics shows behavioral and physiological differences. For performing various manipulations under aquaculture conditions and for experimental purposes, anesthetizing fish can be successfully done using clove oil (Javahery et al 2012), including for different species of catfish (Adeshina et al 2016; Argungu et al 2017). On the other hand, the sedative and anesthetic effect of lavender and wild thyme, proven in fish, must also be taken into account (Mazandarani et al 2017; Yousefi et al 2018; Aydin & Barbas 2020; Boaru et al 2022).

Conclusions. The use of lavender and wild thyme, both separately and in mixture, directly in the fish rearing water did not influence medial water quality parameters. Also, in our experiment, the growth performance and health status of the fish were not influenced. The lavender and wild thyme filters did not change the blood parameters of the exposed fish, being well tolerated by them. There was no influence on the productive parameters as a result of the proven sedative and anesthetic effect of lavender and wild thyme, nor any observable effects on the process of anesthetizing the biological material. Biologically active compounds derived from lavender and thyme are environmentally friendly and can be used in intensive aquaculture as immunostimulators or in the prevention and treatment of some diseases.

Conflict of Interest. The authors declare that there is no conflict of interest.

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