THE EFFECT OF PERACETIC ACID IN THE TREATMENT OF GYRODACTYLOSIS IN RAINBOW TROUT

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Abstract

Gyrodactylosisis a parasitic disease caused by species of genus Gyrodactylus, affecting most species of freshwater fish, characterized by the destruction of skin or rarely the gills.

One of the ectoparasitosis that may cause an increase of mortalities in rainbow trout, is the tegumentary Gyrodactylosis.

This paper studies the effect of administrating peracetic acid in the disinfestation of fish parasitized with Gyrodactylus derjavini Mikailov, 1975.

The chemical substance used for treatment, Divosan Forte (15% peracetic acid), at a dose of 750 ml per day and after seven days of treatment, will achieve a maximum level of efficiency registered by a complete disinfestation.

The correlation between the water chemistry during treatment period and the degree of infestation in trout, shows that the latter varies inversely with the organic substance and directly proportional to the pH of the water.

Successful results advise the use of peracetic acid for treating Gyrodactylosis, in trout, with precautions and recommendations resulting from the presented study.

Keywords: Gyrodactylosis, peracetic acid, rainbow trout

INTRODUCTION

Gyrodactylosis is a parasitic disease caused by species of genus Gyrodactylus, it affects most species of freshwater fish [12] and it is characterized by the destruction of the skin or rarely the gills [10].

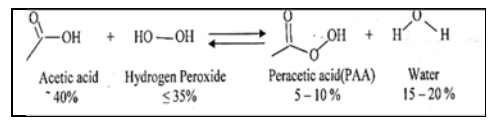
One of the ectoparasitosis that may cause increase mortalities in rainbow trout, is the tegumentary Gyrodactylosis [2].

The species of the genus Gyrodactylus are small sized (less than 1mm) and they are viviparous. The adult parasite contains more embryos and one individual can host in its body up to four successive generations [4], in order to ensure a fast spreading.

In the last years, the identification of new substances for treatments was imposed [11] in order to replace the conventional green malachite (which is carcinogenic and teratogenic) [5]. Researchers have identified that peracetic acid has fast oxidation reaction, which does not generate toxic or adverse effects of residual substance [7].

The use of peracetic acid treatment targets especially trout farmers, which face significant mortalities due to high extensibility degree of parasitic diseases, that are specific to salmonid culture, as well as aplying high fish stocking densities [3]. Eficient disinfestation with non-agresive and non-toxic subtances is obtained by the use of peracetic acid.

Peracetic acid contains acetic acid and hydrogen peroxid, which decomposes into peracetic acid and water according to the reversible chemical reaction shown below [8].



It is present on the market in products from different brands and concentrations, such as Peral-S (5%), Divosan Forte (15%) and Wofasteril 40% [13].

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MATERIAL AND METHOD

This study was conducted in a timeline of 10 days, between 14-23 september 2017, at a trout farm from Neamt county. The degree of *Gyrodactylus derjavini (*Mikailov, 1975) infestation was followed during and after the treatment with peracetic acid. Al well, water chemistry dynamics under the influence of fish disease, treatment and post-treatment was followed and correlated to the infestation degree.

The treatment was conducted in a 7 days period and Divosan Forte (15%) was administered. Daily doses (750 ml) were aplyed, divided into two rounds (morning and evening). The treatment was administered with caution, in order to ensure fish gradual adaptation to the treatment substance.

The parasitic ichthypathological exam was performed on one year old rainbow trout (*Oncorhynchus mykiss* Walb.,1792), with the following biometrics: 279 cm average length and 180 g average weight. The specimen were captured from B3 basin, wich has a water volume of 330 m^3 and 2.700 kg of fish biomass. Aller Bronze granulated fish feed, with a daily ration of 0.8% body per kilogram (maintenance dose) was administered.

Twenty fish samples were examined daily, in order to determine the parasitic degree and disease extensiveness. The presence of other parasites on skin and gills was not observed.

Infested fish with monogenic worms experience the following signs: apathic swimming in the shore area, darkening of the skin, the tendency to rub off the shores and the submerged objects where the parasite is attached, small lesions in the form of ulcerations and tissue necrosis found on the skin and fins, which may be lighter or more severe depending on the intensity of the parasite. Fungal or bacterial attack can be grafted onto these lesions [5].

For diagnosis, the anamnesis, clinical signs and mucus tegumentary scrap, analyzed by microscope, were taken into consideration.

The degree of parasitism of the fish was appreciated by the study of wet mount of tegumentary mucus, between the glass slide and the coverslip with a drop of water and analyzed on the Oxion microscope equipped with the Ccmex3.0MP camcorder and the Olympus stereo microscope of type T2SN 4L00707.

Water chemistry analyzes were performed by standard analysis methods [9] and compared to the maximum admissible values, according to the Order of the Ministry of Environment and Water Management no. 161/2006 concerning the classification of surface water quality in order to determine the ecological status of water bodies [6].

Sludge samples were processed to obtain an aqueous extract (soil / solvent = 1/10) and then specific chemical analysis methods were applied [9].

The determination of pH was made in accordance with SR ISO 10523: 1997, with a laboratory pH meter pH 720 INO Lab with temperature probe.

Determination of chemical oxygen demand was performed according to SR ISO 6060: 1996.

Nitrogen and phosphorus compounds were determined according to standard water and wastewater sampling methods with a DR 2800 spectrophotometer using the LANGE for the water quality kit.

From each pond 3 samples were prelevated that were worked in duplicate.

The statistical analysis was performed using Excel tools. Average values are reported along with standard deviations. The statistical interpretation of data considered differences according to a significance threshold of $P < 0.05$.

RESULTS AND DISCUSSIONS

The taxonomic classification of *Gyrodactylus derjavini (*Mikailov, 1975) parasite is presented in Table no. 1

Table 1

PHYLUM	CLASS	SUBCLASS	ORDER	GENUS	SPECIES
PLATHELMINTHES	MONOGENEA	MONOPISTHOCOTYLEA	GYRODACTYLOIDEA	*Gyrodactylus*	*Gyrodactylus derjavini*

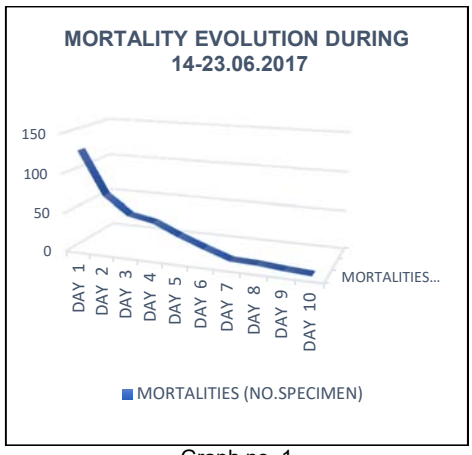
The results regarding the parasitism degree and disease extensiveness are presented in table below.

Table 2

Daily evolution	Parasite name	Affected organ	Infestation degree	Extensivity (%)
Day 1	*Gyrodactylus sp.*	**T**	massive	80
Day 2	*Gyrodactylus sp.*	**T**	massive-medium	40
Day 3	*Gyrodactylus sp.*	**T**	weak-medium	10
Day 4	*Gyrodactylus sp.*	**T**	weak-medium	10
Day 5	*Gyrodactylus sp.*	**T**	weak-accidentally	5
Day 6	*Gyrodactylus sp.*	**T**	accidentally	5
Day 7	*Gyrodactylus sp.*	**T**	accidentally	5
Day 8	*Gyrodactylus sp.*	**T**	0	0
Day 9	*Gyrodactylus sp.*	**T**	-	0
Day 10	*Gyrodactylus sp.*	**T**	-	0

Legend: T=tegument, parasitism degree: week<5 specimens of parasites in the microscopic field, medium between 5-10 parasites in the microscopic field, >10 specimens of parasites in the microscopic field

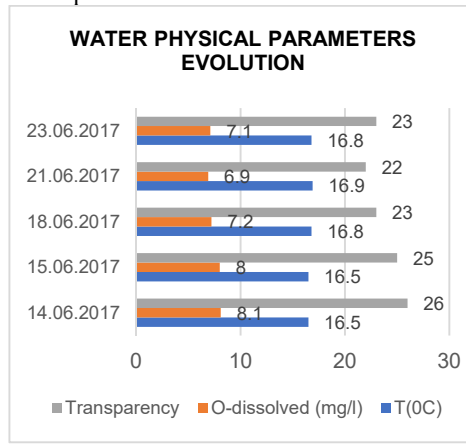
The mortalities recorded at the begining of the experiment, during treatment (15 - 21.06.2017) and post-treatment are shown in Graph 1.



Graph no. 1

Mortality rates declined since the end of the first day of treatment, when the dead fish samples were removed from the basin and keep progressively decreasing until the sixth day of treatment, after which a complete parasite of the fish stock was found.

The physical parameters of the technological water from B3 are represented in Graph no. 2.



Graph no. 2

The hydro chemical analyzes were carried out in a specialized laboratory and revealed a general evolution within the optimum limits of the technologically essential parameters.

The maximum admissible values were not exceeded, except for the organic substance. A slight decrease in the pH was observed towards the end of the experiment, due to the water acidification. Nevertheless, the pH value was within the admitted standards. (Table 3).

Table 3 Chemical parameters of the technological water during study

Physico-chemical determinations / M.U.			DAY				
			14.06.2017 (before treatment)	15.06.2017 (first day of treatment)	18.06.2017 fourth day of treatment)	21.06.2017 (last day of treatment)	23.06.2017 (second day after treatment)
pH		pH	8.72	7.21	7.09	7.01	8.11
Organic substance		Mg MnO_4/l	26.69	62.10	138.40	165.38	110.30
CCO-Mn		mg O_2/l	6.75	6.14	6.83	6.90	6.87
Calcium Ca^{2+}		mg/l	35.19	34.06	36.80	35.20	33.90
Magnezium Mg^{2+}		mg/l	32.43	31.60	31.90	31.50	31.80
Ca^{2+}/Mg^{2+}		raport	1.56	1.65	1.53	1.59	1.58
Total hardness		^0D	12.89	12.19	12.59	12.93	12.52
Ammonia NH_3		mgN/l	0.09	0.13	0.12	0.20	0.19
Amonium	**N-H_4^+**	mgN/l	0.17	0.10	0.18	0.19	0.16
	NH_4^+		0.21	0.20	0.28	1.36	0.29
Nitrite	**N-NO_2^-**	mgN/l	missing	missing	0.001	0.002	0.001
	NO_2^-		missing	missing	0.003	0.006	0.004
Carbonates CO_3^{2+}		mg/l	16.23	17.01	17.91	17.83	17.05
Bicarbonates HCO_3^{2-}		mg/l	90.16	87.96	90.03	88.31	87.01
Total Alkalinity		mgHCl/l	2.05	2.07	2.06	2.09	2.08
Chlorides Cl^-		mg/l	41.05	42.45	42.22	42.94	41.11

Table 4 Average values of hydrochemical parameters extracted from analysis bulletins during treatment compared to the maximum admissible values

Physico-chemical determinations / M.U.			Average value (during 7 days of treatment)	Maximum admitted level (Ord.nr. 161/2006)	Maximum admitted level (literature)
pH	upH		7.10	6.5-8.5	
Organic substance	MgKMnO_4/l		121.96	-	60
CCO-Mn	mg O_2 /l		6.62	10	15
Calcium Ca^{2+}	mg/l		35.35	100	160
Magnezium Mg^{2+}	mg/l		31.66	50	50
Ca^{2+}/ Mg^{2+}	raport		1.59	-	5
Total hardness	^0D		12.57	-	20
Ammonia NH_3	mgN/l		0.17	-	0.2
Amonium	N - NH_4^+	mgN/l	0.15	-	-
	NH_4^+		0.61	-	2
Nitrites	N - NO_2^-	mgN/l	0.001	0.03	-
	NO_2^-		0.004	-	0.2
Carbonates CO_3^{2-}	mg/l		17.58	-	20
Bicarbonates HCO_3^-	mg/l		88.76	-	600
Total Alkalinity	mgHCl/l		2.07	-	6
Chlorides Cl^-	mg/l		42.49	50	40

The obtained data shows a progressive increase in the amount of organic substance during the treatment from 62.10 to 165.38 mgKMnO4/l [8]. Also, a decrease in the pH from 7.21 to 7.01 pH was observed, due to the effect of peracetic acid used in the treatment of Gyrodactylosis.

The increase of organic substance in the technological water is explained by the distruction of the studied parasites and other groups of aquatic organisms (bacteria, algae, zooplanktons, fungi) [13]. Decomposition of accumulated dead organisms in the water is influenced directly and indirectly (through the activity of decaying bacteria) and this phenomenon cumulated with the decrease of the pH units due to the peracetic acid, influence the amount of oxygen dissolved in the water.

During treatment, the water temperature varied between 16.5-16.90^{0}C, inversely proportional to the dissolved oxygen (8.0-6.9 mg/l). A decrease in water transparency (25-22cm), where the necessary light supports

phytoplanktonic photosynthesis processes, determines a decreases of the dissolved oxygen in water. In trout farms during rainy or thawing periods, the water gets a strongly acidic pH and the transparency reaches less than 8 cm on the Secchi disc.

The water temperature varies directly proportional with the oxidation rate of organic substances and the salt solubility, therefore in the summer season water stratification occurs, heating the surface layer. During the day oxygen deficiency manifests in the surface layer and during night the water cools down and descends to the bottom of the basin.

Sediments contain large amounts of organic substances that consume a lot of oxygen, creating a deficiency, which leads to increase ammonia concentration (0.13-0.20mgN/l).

From the point of view of the disinfestation efficiency, an evolution is observed, which reaches an ascending maximum on the sixth day of treatment and continues constantly until the end of the experiment.

The evolution process of disinfestation with peracetic acid, according to water chemistry, expressed in the present study, highlights the effectiveness of its use but also the limits of its use, with the rigorous precautions deriving from the physico-chemical parameters of the water.

CONCLUSIONS

Treatment effectiveness of Gyrodactylosis with peracetic acid of rainbow trout is supported by good results regarding the degree of disinfestation at the end of the treatment and after its completion.

Evolution of water chemical parameters records slightly increasing values during treatment for organic matter and decreases in pH units.

These changes, cumulated with the increase in water temperature and decrease in the amount of dissolved oxygen, may negatively influence the chemistry of the technological water, therefore it is recommended that Divosan Forte should be administered (halved the dose into 2 rounds, in the morning and in the evening) 7 days for total disinfestation. The treatment can be resumed after a 10-day break depending on the parasite life cycle and the probability of re-infesting the fish.

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