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TREATMENT OF *CYPRINUS CARPIO* L. AND *CLARIAS GARIEPINUS* (BURCHELL) EMBRYOS WITH FORMALIN AND MALACHITE GREEN: EFFECT OF CONCENTRATION AND LENGTH OF TREATMENT ON THEIR SURVIVAL

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ABSTRACT

THERON, J., PRINSLOO, J. F. & SCHOONBEE, H. J., 1991. Treatment of *Cyprinus carpio* L. and *Clarias gariepinus* (Burchell) embryos with formalin and malachite green: effect of concentration and length of treatment on their survival. *Onderstepoort Journal of Veterinary Research,* 58, 239–243 (1991).

Malachite green and formalin were investigated as prophylactic dip treatments for developing embryos of *Cyprinus carpio* and *Clarias gariepinus*. Based on the findings, recommendations are made on the respective concentrations to be used and exposure periods to be followed for both formalin and malachite green for both fish species.

INTRODUCTION

Aquaculture is at present an important and rapidly expanding industry in many parts of the world, as attempts are made to supplement the dwindling supplies of natural fish resources from the sea and inland waters (Anon., 1986; Rhodes, 1987; Liao, 1988).

In South Africa only a few marine fin- and shellfish species are exploited commercially by means of aquaculture (Safriel & Bruton, 1984). In the case of freshwater fish, it is only the rainbow trout *Oncorhynchus mykiss* which is cultivated on a large scale for the local market. Among the warmwater fish, the European common carp was studied for its aquaculture potential during the early 1950's (Lombard, 1961; Kruger, 1975). Prinsloo & Schoonbee (1984a, b, c; 1987a, b, c) also demonstrated the value of this fish when used in polyculture, using agricultural wastes to supplement its diet of formulated feed. Recently, a number of investigations were made locally on the induced spawning and rearing of the larvae of the sharptooth catfish *Clarias gariepinus* (Schoonbee, Hecht, Polling & Saayman, 1980; Uys & Hecht, 1985; Polling, Van der Waal & Schoonbee, 1987; Polling, Schoonbee, Prinsloo & Wiid, 1988; Hecht, 1989). This fish is now pond-produced commercially by a number of farmers in the Transvaal and elsewhere in the country. Indications are that with the necessary further market development the catfish industry may well become an important factor in aquaculture in South Africa.

With the large scale production of *C. gariepinus* juveniles, problems arose with occasional mass mortalities caused by fungal and protozoal infections such as *Saprolegnia* spp., *Ichthyophthirius multifiliis* and *Trichodina* spp. This seriously affected the supply of juveniles of the sharptooth catfish to the local fish farmer. The present investigation was, therefore, aimed at overcoming this problem by investigating the possible sensitivity of embryos of *C. gariepinus* to various concentration levels of formalin and malachite green in view of the future use of these medicaments during the large scale outbreaks of diseases in the course of hatchery operations. The embroys of *C. carpio* were also subjected to the same type of treatments for the purpose of comparison.

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MATERIALS AND METHODS

Experimental design

Research conducted on the prophylactic treatment of embryos of both species was done in 10 ℓ glass containers. Three such aquariums were placed in a larger tank containing temperature controlled water. Each set of aquariums was illuminated by means of a 40 W fluorescent light.

Filtered municipal water was used after maturation for a minimum period of 2 days in a 250 ℓ aerated tank. Water temperature in this tank was maintained at 28 ±1,0 °C, using thermostatically controlled heaters. Oil-free, diffused air was supplied to water in both the maturation tank and the experimental aquariums.

Fish embryo hatching trays used in this study were of an experimental design, consisting of 90 × 140 mm Perspex frames, covered with nylon screens of mesh sizes of 950 µm and 540 µm. These trays were used to hold the embryos of both *C. carpio* and *C. gariepinus,* respectively.

Spawners of both species were collected from broodstock ponds at the Turfloop Fish Breeding Station from where they were transferred to the hatchery. Spawning techniques followed were according to methods developed by Schoonbee *et al.* (1980), Hecht, Saayman & Polling (1982) and Polling *et al.* (1987). Within 10 min following fertilization, eggs of both species were deposited on the screens of hatching trays. Egg deposition occurred under water in order to facilitate adhesion of the eggs onto the screens. Before adhesion became effective, the eggs were evenly dispersed over the screens in a monolayer, using a small soft brush. Randomly selected trays containing the developing embryos were transferred in triplicate to individual aquariums for prophylactic treatment.

Dip treatment containers holding accurately measured concentrations of malachite green or formalin (Table 1) were prepared immediately before the commencement of the experiment. In the case of *C. carpio* which has a more adhesive egg than *C.*

TABLE 1 Concentrations used and duration of dip treatment followed for *Cyprinus carpio* and *Clarias gariepinus* embryos

Chemical	Concentration mg/ℓ	Duration of treatment s (malachite green) min (formalin)				
Malachite green	750			10	20	40
	1 500	5	10	15	30	60
Formalin	2 000	5	10	15	20	30

TABLE 2 Selected physical and chemical parameters of acclimated heated (25 ±1,0 °C) and aerated water used in the hatching of melachite green and formalin treated *Clarias gariepinus* embryos, on horizontal trays, in 10,0 ℓ aquariums. Samples (n=6) taken immediately before introduction of the fertilized eggs and then at 6 and 24 h after introduction, respectively

Analysis		Acclimated water (n=6) x̄	Acclimated water (n=6) Range	6 h after introduction of eggs (n=6) x̄	6 h after introduction of eggs (n=6) Range	24 h after introduction of eggs (n=6) x̄	24 h after introduction of eggs (n=6) Range
Conductivity µS/cm		80	78 – 84	87,1	87,0 – 87,3	92,7	92,2 – 93,2
pH			7,38 – 7,43		7,66 – 7,68		7,53 – 7,55
Dissolved oxygen	Expressed in mg/ℓ	6,4	5,9 – 6,7	6,9	6,9 – 6,9	6,8	6,6 – 6,9
Nitrate (NO_3)		0,44	0,37 – 0,49	2,86	2,64 – 3,08	2,2	2,2 – 2,2
Nitrite (NO_2)		0,023	0,020 – 0,032	0,025	0,023 – 0,026	0,045	0,040 – 0,050
Ammonia (NH_3)		0,037	0,030 – 0,041	0,085	0,080 – 0,087	0,312	0,281 – 0,342
Phosphate (PO_4)		0,150	0,146 – 0,161				
Alkalinity as $CaCO_3$		18	17 – 20				
Calcium hardness as $CaCO_3$		16	15 – 18				
Total hardness as $CaCO_3$		20	18 – 21				

TABLE 3 The effects of selected concentrations of malachite green and formalin, as well as different treatment periods, on the survival of *Cyprinus carpio* embryos. Each treatment done in triplicate. Water temperature kept at 25 ± 1,0 °C

Treatment type and concentration	Duration of treatment	0 h	25 h post-fertilization Mean % live embryos ± S.D. n = 9	30 h post-fertilization	38 h post-fertilization Mean % live embryos ± S.D. n = 9	Indications of increase in the mortality rate after treatment[a]	Development of fungi
Malachite green 750 mg/ℓ	10 s	Fertilization	78,8 ± 4,49	Treatment	76,5 ± 7,61	0	No
	20 s		76,1 ± 6,12		73,3 ± 5,17	0	No
	40 s		71,8 ± 3,54		72,4 ± 13,12	0	No
Malachite green 1 500 mg/ℓ	5 s		59,8 ± 9,38		64,7 ± 8,67	0	Yes from 50 h
	10 s		63,4 ± 12,79		68,2 ± 2,25	0	No
	15 s		67,1 ± 9,83		68,5 ± 10,32	0	No
	30 s		72,2 ± 5,05		72,6 ± 8,28	0	No
	60 s		72,0 ± 5,44		31,8 ± 19,81	+++	No
Formalin 2 000 mg/ℓ	5 min		75,2 ± 2,20		72,8 ± 7,97	0	No
	10 min		67,3 ± 7,64		75,6 ± 3,21	0	No
	15 min		68,9 ± 13,39		74,7 ± 7,21	0	No
	20 min		77,2 ± 6,23		78,9 ± 6,27	0	No
	30 min		82,6 ± 3,89		76,5 ± 7,90	0	No
Control	30 min		68,1 ± 10,56		66,6 ± 5,64	0	No
	30 min		77,6 ± 4,67		81,1 ± 2,91	0	Yes from 38 h

[a]0 No noticeable increase in mortalities after treatment
\+ Slight increase in mortalities after treatment
++ Noticeable increase in mortalities after treament
+++ Definite increase in mortalities after treatment
++++ Very definite increase in mortalities after treatment
S.D. = Standard deviation

gariepinus, triplicate sets of trays, holding the embryos, were suspended vertically in the aquariums. During treatment each set of trays was gently lifted from the aquariums and lowered for the specified period of exposure into the appropriate concentration of malachite green or formalin (Table 1). After treatment, the sets of trays were carefully rinsed in clean acclimated and aerated tap water kept at 25 ±1 °C. The trays were then returned to post-treatment holding aquariums where further development and the eventual hatching of embryos were observed. Procedures followed in the prophylactic treatment of *C. gariepinus* embryos were essentially the same, the major difference being the horizontal position of the trays because of the generally low glutinosity of the fertilized eggs. These sets of trays were gently lowered horizontally and removed from the respective solutions to prevent unnecessary loss of the developing *C. gariepinus* embryos.

As controls, 2 seperate sets of 3 trays, each containing the embryos of each species, were allowed to develop in clean acclimated water without any treatment.

Microscopic examination of treated and untreated embryos on the hatching trays

Treated and untreated embryos of both species were examined under a stereo microscope for evaluation of mortalities until hatching took place. Because of the differences in hatching time between the two species, the embryos of *C. carpio* were inspected for survival at 25 h and 38 h after fertilization, respectively. In *C. gariepinus* inspection of egg survival was made once at 23 h following fertilization.

After hatching, the larvae were observed for a further 6 days in the same aquariums in which they were hatched to observe any possible signs of mortalities and deformities which could have been caused by the different treatments with formalin or malachite green.

At 6 h and 24 h after introduction, analysis of selected chemical parameters (Table 2) was done according to APHA (1980) on the acclimated water, as well as on the water into which the treated embryos were introduced.

TABLE 4 The effects of selected concentrations of malachite green and formalin, as well as different treatment periods on the survival of *Clarias gariepinus* embryos. Each treatment done in triplicate. Water temperature kept at 25 ±1,0 °C

Treatment type and concentration	Duration of treatment	0 h	14 h post-fertilization Mean % live embryos ±S.D. n = 20	16 h post-fertilization	23 h post-fertilization Mean % live embryos ±S.D. n = 9	Indications of increase in the mortality rate after treatment[a]	Development of fungi
Malachite green 750 mg/ℓ	10 s	Fertilization		Treatment	51,4 ± 6,16	+	No
	20 s				*	*	No
	40 s				*	*	No
Malachite green 1 500 mg/ℓ	5 s		60,6 ± 9,48		59,9 ± 6,88	0	No
	10 s		Only one set of 20		47,4 ± 10,86	++	No
	15 s		different counts was		42,8 ± 9,61	++	No
	30 s		made on randomly		51,8 ± 6,17	+	No
	60 s		selected areas of the trays to obtain this figure which was taken as representative of the entire batch of embryos before treatment		21,2 ± 6,18	++++	No
Formalin 2 000 mg/ℓ	5 min				53,9 ± 9,86	+	No
	10 min				52,4 ± 2,75	+	No
	15 min				47,3 ± 6,61	++	No
	20 min				49,3 ± 8,43	++	No
	30 min				51,3 ± 6,89	+	No
Control	30 min				62,4 ± 8,70	0	No
	30 min				60,4 ± 7,50	0	No

[a]* Hatching commenced before counting was finished
0 No noticeable increase in mortalities after treatment
+ Slight increase in mortalities after treatment
++ Noticeable increase in mortalities after treatment
+++ Definite increase in mortalities after treatment
++++ Very definite increase in mortalities after treatment
S.D. = Standard deviation

Values for alkalinity, calcium and total hardness were only determined on the acclimated water in order to obtain an assessment of the levels of these parameters in the stock water used.

RESULTS

Results of the chemical analysis of the acclimated water in the aquariums before the introduction of the fertilized *C. gariepinus* eggs, and at 6 and 24 h after introduction, are summarised in Table 2.

There was an almost 16 % increase in the conductivity of the water from an initial mean of 80 µS/cm, to more than 92 µS/cm, within a period of 24 h. Concurrently, there was an increase in nitrate and in particular a build-up of ammonia after 24 h. Dissolved oxygen values were, however, maintained at approximately the same level throughout the experiment. The pH of the water remained fairly constant between 7 and 8.

Values for alkalinity, calcium and total hardness showed that the water used in the experiments was clearly alkaline with moderate hardness levels.

Effects of malachite green and formalin on the embryos of C. carpio *and* C. gariepinus

The effects of the 2 concentrations of malachite green, namely 750 and 1 500 mg/ℓ, and a concentration of 2 000 mg/ℓ of formalin, applied for different treatment periods, on the survival of the embryos of *C. carpio* and *C. gariepinus* are summarised in Tables 3 and 4 respectively.

In the case of *C. carpio* embryos exposed to a concentration of 750 mg/ℓ malachite green (Table 3), no marked difference was observed after 25 and 38 h, between the results obtained for the control (untreated) and treated groups of embryos. The survival rate of embryos remained virtually the same after 25 and 38 h post-fertilization, respectively. In the case of the 1 500 mg/ℓ malachite green concentrations, the survival rate of the embryos was generally slightly lower for most exposure periods compared to those at 750 mg/ℓ, with no consistent pattern of survival with an increase in exposure times. There was 1 exception, however, namely the 60 s exposure period to 1 500 mg/ℓ malachite green, which showed a marked decline in embryo survival (31,8 %), 38 h after fertilization.

Where 2 000 mg/ℓ formalin was used, this chemical had no marked effect on the survival of the embryos up to 38 h post-fertilization for any of the exposure periods. In fact, survival after longer periods of exposure time appeared to be even better than in some of the control experiments.

Results on the effects of the different concentrations of malachite green on the survival of the embryos of *C. gariepinus* 23 h after fertilization showed a generally lower survival rate of the embryos than was the case for *C. carpio* at approximately the same post-fertilization period. There was no real difference in the survival of embryos exposed to 750 mg/ℓ for 10 s and those exposed to 1 500 mg/ℓ malachite green for 5 to 30 s. It was only after a 60 s exposure period that there was a dramatic decline in the survival of the embryos (21,2 %) (Table 4). The survival of *C. gariepinus* embryos exposed to 2 000 mg/ℓ formalin for 5–30 min periods, did not show any marked deterioration with increase in exposure time. In this case the percentage survival was very much the same as that recorded for the 5 to 30 s exposures to 1 500 mg/ℓ concentration of malachite green. Survival values were found to be slightly higher on average than in the other experimental groups.

Observation showed that only in 2 instances did fungi develop on the *C. carpio* embryos before hatching took place (Table 3). Because of the much shorter period of embryonic development of *C. gariepinus*, there were no signs of any fungal development.

DISCUSSION

The results obtained in the present investigation substantiate the procedures for formalin treatment of fungi on fish eggs as listed in Hoffman & Meyer (1974). The present results showed that *C. carpio* embryos survive well when they are subjected to

2 000 mg/ℓ formalin for the recommended 15-min exposure period. A high survival rate was obtained, even when the embryos were exposed to this chemical for 30 min.

C. carpio embryos reacted in much the same way to a malachite green treatment (1 500 mg/ℓ for 10 s), as the channel catfish *Ictalurus punctatus* embryos (Hoffman & Meyer, 1974). Good survival rates were still obtained with carp, using this concentration for exposure periods of up to 30 s. In commercial enterprises this may have the obvious advantage of tripling the safe exposure period.

A comparison of resulsts obtained for *C. carpio* and *C. gariepinus* embryos treated with the same concentrations of malachite green and formalin and subjected to the same exposure times showed the embryos of *C. gariepinus* to be much more sensitive to both these chemicals than was the case for the embryos of *C. carpio*. These findings support the warning expressed by Hoffman & Meyer (1974), that extreme caution must be exercised when using a prophylactic agent in the treatment of fish, as the species and even the developmental stages during treatment may determine the sensitivity to the chemical (Wedemeyer, 1971).

It is known from the literature that prophylactic treatment of fish embryos with chemical agents may cause adverse side-effects, such as chromosomal aberrations (Steffens, Lieder, Nehring & Hattop, 1961), and delayed hatching times (Meyer & Jorgenson, 1983). The latter authors reported a delay of 8 d in the hatching time of trout embryos after 15 applications of malachite green at a concentration of 3 mg/ℓ for 1 h administered every alternate day. However, 5 mg/ℓ applied at weekly intervals (5 applications), caused a delay in hatching time of only 5 d. It is thus evident that the delayed hatching time could have been caused by the cumulative effect of the frequency of malachite green applications. Meyer & Jorgenson (1983) also reported that all the treated groups exhibited variable percentages for a number of deformities, as well as the occurrence of retarded growth 3 weeks after hatching. No such delay in hatching time was observed for the carp and catfish embryos in the present investigation. This may perhaps be explained by the fact that Meyer & Jorgenson (1983) worked with coldwater fish, whereas the embryos of *C. carpio* and *C. gariepinus* were incubated at a constant water temperature of 25 ±1,0 °C. The embryonic developmental time of a maximum of 60 h in the case of *C. carpio* was also much shorter (hours instead of weeks) than in the case of coldwater fish.

It can thus be stated that *C. carpio* embryos can safely be exposed to a 750 mg/ℓ concentration of malachite green for periods of up to 40 s under the environmental and water quality conditions in which the present series of investigations was conducted. A 1 500 mg/ℓ malachite green treatment is also safe for exposure periods of up to 30 s. Hoffman & Meyer (1974) list a 10 s exposure period at this latter concentration of malachite green as effective against fungal infestations of fish embryos.

Hoffman & Meyer (1974) demonstrated that exposure for 15 min to formalin at a concentration of 2 000 mg/ℓ is effective against fungal infestations of fish eggs. The *C. carpio* embryos in the present study could, however, withstand a concentration of 2 000 mg/ℓ formalin for periods of up to 90 min, without any apparent negative after-effects. There is, therefore, also a large margin of safety when formalin is used on *C. carpio* embryos at this concentration.

Results further showed that *C. gariepinus* embryos are more sensitive to both formalin and malachite green treatments at any of the concentrations used in the present experiment. One way of overcoming possible fungal infections of the embryos without using chemical treatment would be to increase the water temperatures to 26–28 °C, which would facilitate the embryonic rate of development and shorten the hatching time of this species.

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