

## **Minimum inhibitory concentration (MIC-test) of formalin for *Dactylogyrus minutus* Kulwièc, 1927 (Monogenea: Dactylogyridae): *in vitro* test**

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The formaldehyde has been widely used in its diluted form as an alternative to malachite green in the treatment of parasites and fungi. It has been used as anti-fungal and against parasites, especially protozoans and monogeneans from the gills and body surface of fish and immature amphibians like tadpoles. The minimum inhibitory concentration (MIC-test) of formalin *in vitro* that controls the *Dactylogyrus minutus* Kulwièc, 1927 of *Cyprinus carpio* var. *koi* was determined in this study. Samples of carp var. *koi* (1.73±0.25 g mean weight and 4.7±0.46 cm total length) were obtained in September 2016 from a fish farm located in Biguaçu, Santa Catarina, Brazil and transported live to Aquatic Organisms Health Laboratory. Eight concentrations of formalin were tested: 50, 75, 100, 125, 150, 175, 200, 250 ppm. In addition, two controls were prepared, one with water from the recirculation system and another with distilled water. Gill filaments from parasitized gill arches were collected to obtain the adult specimens of *D. minutus*. Parasites were placed into 6-well flat plates for cell culture in a volume of 3 ml per well, where each set of parasitized filaments received a formalin concentration. Mortality observations were recorded every 5 minutes at the highest concentrations or 10 minutes at the lowest concentrations using a stereomicroscope. The parasites were considered dead, when the absence of movements to the stimulus with needle was verified, as well as typical characteristics of mortality as the wrinkling of the body. Data were submitted to analysis of variance (ANOVA) and Tukey's test was used to compare means ( $p < 0.05$ ). The concentration of 200 ppm was the most lethal to *D. minutus*, killing all the parasites in 16 minutes. However, for 250, 175, 125 and 75 ppm there was no statistical difference when compared to the two most lethal concentrations. The concentration of 100 ppm took longer to act on all monogeneans, however the time (47 minutes) was not higher than the time normally recommended in fish treatment. As some protocols indicate baths of at least 1 hour or even continuous baths, with this study, we could guarantee the real efficacy of the formalin directly in the parasite by monitoring the time and concentration.

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