

THE EFFECT OF MALACHITE GREEN AS A FUNGICIDE

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ABSTRACT

A series of tests with malachite green, used at various concentrations and treatment durations, were made on six species of water molds known to be parasitic on fish and/or fish eggs. The tests indicate that these species have markedly different tolerances to the dye. While a treatment of as little as 1 ppm of malachite green for five minutes was effective for *Saprolegnia parasitica*, it took 10 ppm for five minutes to control *Achlya ambisexualis* and *Allomyces macrogynus*, and 15 ppm for one hour to control *Achlya oblongata*.

INTRODUCTION

The occurrence of parasitic fungi constitutes a chronic problem in fish hatcheries and aquaria. Fish suffering from stress and injury may become infected by fungi, resulting in considerable economic loss, particularly where valuable aquarium and cultured fish are involved. Nigrelli (1943) reported that over 45 deaths in one year were incurred among fishes at the New York Aquarium due to infestations by unidentified species of the genus *Saprolegnia*. Many fish hatcheries have serious problems with fungal infections of fish and fish eggs (Glen L. Hoffman, 196× personal communication).

The most common treatment for parasitized fish and fish eggs is malachite green oxalate. This aniline dye is used as a dip or flush to eliminate or prevent the growth of fungi. Many studies have been made to try and determine the most effective concentrations for fungicidal-fungistatic effects. Prominent among them is that of O'Donnell (1941), who found that a 10-30-second dip in 67 ppm (1:15,000) malachite green was effective and non-toxic to 18 species of fish. Johnson *et al.*, (1955) found that semi-weekly treatments of malachite green at 5 ppm for one hour eliminated losses among fry and eggs due to fungi.

It is known that the members of at least six genera of "water mold" fungi are natural parasites of fish and/or fish eggs (Scott and O'Bier, 1962), including *Saprolegnia*, *Achlya*, *Aphanomyces*, *Pythium*, *Leptomitus*, and *Allomyces*. It is possible, however, that there are differences in resistance to malachite green among various species within these genera. It was the purpose of this investigation to examine the usefulness of malachite green as a fungicide to six species of fungi and to assay its relative toxicity to them.

MATERIALS AND METHODS

Six species of fungi, differing in their ability to kill or damage fish were tested. These species were *Saprolegnia parasitica*, *S. ferax*, *Achlya oblongata*, *A. racemosa*, and both male and female strains of *A. ambisexualis*, all of which belong to the Saprolegniaceae, and *Allomyces macrogynus* of the Blastocladiaceae.

The seven kinds of fungi were tested with malachite green in the following manner. Sterile petri dishes containing 20 ml of 1, 5, 10, and 15 ppm solutions of malachite green were used in each test. A sesame seed bearing a flourishing growth of mycelium with zoosporengia was placed into the dish of test solution for 5, 15, and 30 minutes, and 1, 12, and 24 hours. The fungal colony was then removed and rinsed twice with double distilled water before being placed in a new petri dish containing charcoal-filtered tap water and a number of boiled sesame seeds. The test fungi were incubated for seven days in the new petri dish at a controlled temperature of 22°C and under light conditions of 15 hours of indirect light alternating with nine hours of darkness during each 24-hour period, in an effort to simulate natural conditions. If, after seven days, the fungus had not infested the new seeds, the test was terminated and the time/concentration considered to be effective. Each test was duplicated.

RESULTS

The results of these duplicate experiments to determine the effectiveness of malachite green as a fungicide against the seven selected water molds are summarized in Table 1. From this it is apparent that the effects of this chemical were different for different species of fungi.

Saprolegnia parasitica was prevented from infesting new seeds by as little as 1 ppm for five minutes. *Saprolegnia ferax* showed similar results, although a slightly higher concentration of malachite green—5 ppm—was necessary to prevent subsequent growth on new sesame seeds; it was still capable of propagation when treated with only 1 ppm of malachite green for five minutes. *Achlya oblongata* was the most resistant to malachite green of all species of fungi studied and continued to be capable of propagation of new seeds until the treatment was increased to 15 ppm for one hour. *Achlya racemosa* was effectively prevented from propagation by treatment with 5 ppm for five minutes. *Achlya ambisexualis* (both sexes) and *Allomyces macrogynus* became incapable of propagating on new seeds after immersion in 10 ppm of malachite green for five minutes.

TABLE 1

Growth of fungus after treatment with malachite green at indicated combinations of concentration and exposure time

	Time ppm	5 min.				15 min.				30 min.			
		1	5	10	15	1	5	10	15	1	5	10	15
<i>Saprolegnia parasitica</i>		O	O	O	O	O	O	O	O	O	O	O	O
<i>S. ferax</i>		X	O	O	O	O	O	O	O	O	O	O	O
<i>Achlya oblongata</i>		X	X	X	X	X	X	X	X	X	X	X	X
<i>A. ambisexualis</i> (m)		X	X	O	O	X	O	O	O	X	O	O	O
<i>A. ambisexualis</i> (f)		X	O	O	O	X	O	O	O	X	O	O	O
<i>A. racemosa</i>		X	O	O	O	X	O	O	O	X	O	O	O
<i>Allomyces macrogynus</i>		X	X	O	O	X	O	O	O	X	O	O	O

	Time ppm	1 hr				12 hrs				24 hrs			
		1	5	10	15	1	5	10	15	1	5	10	15
<i>Saprolegnia parasitica</i>		O	O	O	O	O	O	O	O	O	O	O	O
<i>S. ferax</i>		O	O	O	O	O	O	O	O	O	O	O	O
<i>Achlya oblongata</i>		X	X	X	O	X	X	O	O	X	X	O	O
<i>A. ambisexualis</i> (m)		X	O	O	O	O	O	O	O	O	O	O	O
<i>A. ambisexualis</i> (f)		X	O	O	O	X	O	O	O	X	O	O	O
<i>A. racemosa</i>		O	O	O	O	O	O	O	O	O	O	O	O
<i>Allomyces macrogynus</i>		X	O	O	O	X	O	O	O	X	O	O	O

Controls used in each test exhibited growth in from 48-72 hours. Each test was duplicated.

Symbols: O—no growth
X—growth within 7 days
(m)—male (f)—female

DISCUSSION

Fish and fish eggs are probably not immune to infection, with exposure to 15 parts of malachite green oxalate for only up to 30 minutes, because the tests indicate that at least one species, *Achlya oblongata*, is still capable of infecting new substrates at that concentration. Exposures of 24 hours at 5 ppm are sufficient to control all fungi tested in this study. It should be noted that malachite green is extremely toxic to *Saprolegnia parasitica*, as this species is unable to infest new seeds when treated with as little as 1 ppm for one minute. This undoubtedly

explains why malachite green is effective in controlling certain fungus infections, many of which are due to this particular fungus (Scott and O'Bier, 1962). It must be stressed that, if malachite green is to be used, the fungus causing the infection should be identified to species so as to determine the nature of adequate treatment. According to Arasaki *et al.* (1958), as little as 0.02 ppm of malachite green will restrain the growth of saprolegniaceous hyphae and sporangia. Scott and Warren (1964) reported that 2 ppm of malachite green, when applied to a fungus for 24 hours, would stop all growth of *Saprolegnia* sp., *Achlya americana*, and *Pythium* sp. High concentrations, however, when applied for only short periods of time are not completely effective (table 1).

O'Donnell (1941) recommends a treatment of 10 to 30 seconds in a 67-ppm solution as being non-toxic to 18 species of fish and yet effective as a fungicide. This concentration, tested by the author on seven species of fungi for 20 seconds, was found to be toxic to all except *A. ambisexualis* (male), which did infest new seeds after four days. Quite possibly, therefore, other species of fungi may also be refractory to this treatment.

When fish are exposed for longer periods, even to much lesser concentrations, malachite green proves highly toxic to the fish. Willford (1967) reports that bluegills, and rainbow, brook, brown, and lake trout show 50% mortality when treated with concentrations of 0.6 ppm or less at 12°C. At 17°C, the 24-hr LC₅₀ (lethal concentration at which 50% of the test fish die) for channel catfish was 0.21 ppm.

It would appear that, if absolute control of parasitic fungi is to be obtained with long term (12-24 hr) treatments, concentrations that are lethal to the fish would have to be used. If higher concentrations and shorter treatment durations are used, the treatment may not be effective in controlling all species of fungi. At best malachite green is an imperfect tool with limited value and should be used only where identification of the fungus species present shows that, at certain specific concentrations and time intervals, the method would be effective.

Some promising results in the control of parasitic fungi have already been obtained with ozone (Benoit and Matlin, 1966), and another aniline dye, acriflavine (Martin, 1967), though the latter must be cleared by the Food and Drug Administration before it can be used to treat fishes. Further investigations with this and other as yet untested compounds could, in the near future, make the use of malachite green obsolete.

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LITERATURE CITED

- Agersborg, H. P. Kjershow. 1933. Salient problems in the artificial rearing of salmonid fishes with special reference to intestinal fungisitis and the cause of white-spot disease. *Trans. of the Amer. Fisheries Soc.*, 63: 242.
- Arasaki, S. K., K. Nozawa, and M. Mizaki. 1958. On the pathogenicity of water mold. *Bull. Jap. Fisheries Soc.*, 23: 593-598 as seen in Fishery Leaflet 564, Bureau of Sport Fisheries and Wildlife.
- Benoit, R. F. and N. A. Matlin. 1966. Control of *Saprolegnia* on eggs of rainbow trout (*Salmo gairdneri*) with ozone. *Trans. of the Amer. Fisheries Soc.*, 95(4): 430-432.

- Johnson, H. E., C. D. Adams, and R. J. McElrath.** 1955. A new method of treating salmon eggs and fry with malachite green. *Prog. Fish-Cult.* 17(1): 76-78.
- O'Donnell, John.** 1941. A new method of combating fungus infection. *Prog. Fish-Cult.* (56): 18-20.
- Martin, Richard L.** 1967. A comparative investigation of the effect of various concentrations of malachite green and of acriflavine on fungi associated with diseased fish. Unpublished M.Sc. thesis, The Ohio State University. 000 p.
- Nigrelli, R. F.** 1943. Causes of diseases and death of fishes in captivity. *Zoologica*, 28: 203-216.
- Scott, Wm., and A. H. O'Bier.** 1962. Aquatic fungi associated with diseased fish and fish eggs. *Prog. Fish-Cult.* 24(1): 3-15.
- Scott, W. W., and C. O. Warren, Jr.** 1964. Studies of the host range and chemical control of fungi associated with diseased tropical fish. Va. Agric. Exper. Sta., Blacksburg, Va. Tech. Bull. 171.
- Vishniac, H. S., and R. F. Nigrelli.** 1957. The ability of Saprolegniaceae to parasitize platyfish. *Zoologica*, 42(4): 131-134.
- Willford, W. A.** 1967. Investigations in fish control. The toxicity of 22 therapeutic compounds to six fishes. Bureau of Sport Fisheries and Wildlife Resource Publication (In Press).
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