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NOTES

Evaluation of Several Commercial Algicides for Control of Odor-producing Cyanobacteria

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INTRODUCTION

The production of certain odorous metabolites is an undesirable attribute of cyanobacteria (blue-green algae) growth in aquaculture ponds [e.g., channel catfish (*Ictalurus punctatus*)] and in drinking water reservoirs. The most common odorous compounds encountered in catfish aquaculture are geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) and 2-methylisoborneol (*exo*-1,2,7,7-tetramethylbicyclo[2.2.1]heptan-2-ol). These compounds are also frequently encountered worldwide in reservoirs and aqueducts used for municipal drinking water systems (Schrader et al. 2002). Geosmin is typically described as having an "earthy" odor while 2-methylisoborneol (MIB) is referred to as "musty." The uptake of these compounds by catfish occurs mainly across the gill membranes and can taint the flesh causing them to be unpalatable and subsequently unmarketable. Catfish producers must hold the catfish in ponds until they lose the earthy and/or musty "off-flavor" which can take weeks or months. These delays in harvest have been estimated to cost producers as much as $30 million annually (Schrader et al. 2003). Earthy and musty off-flavors in municipal drinking water systems are also costly due to additional management expenses in order to remove the off-flavor compounds (e.g., carbon filtration or ozone treatments) or preventive treatments (e.g., application of algicides).

The most common management approach in catfish aquaculture to control earthy and musty off-flavors is the use of algicides. The application of algicides may initially make the off-flavor episode more intense since damaged and dying cyanobacterial cells will release intracellular stores of geosmin or MIB (Peterson et al. 1995). Currently, only copper-based products (e.g., chelated-copper products and copper sulfate) have United States Environmental Protection Agency (USEPA) approval for use in catfish aquaculture ponds and municipal drinking water systems for the management of earthy/musty off-flavors. Diuron [*N*'-(3,4-dichlorophenyl)-*N*, *N*-dimethylurea], under section 18 emergency exemption permission by the USEPA for management of MIB-related off-flavors in catfish, must be approved annually and future approvals are not guaranteed. These synthetic algicides have several negative attributes including broad-spectrum toxicity towards phytoplankton, persistence in the environment, and the public's negative perception to the use of synthetic compounds in food-fish production ponds and municipal drinking water systems.

Several new products, some of which are natural-based, have become available commercially that may be useful as selective algicides in managing off-flavor producing cyanobacteria. In this study, several of these algicides were evaluated using a rapid bioassay to determine their effectiveness in controlling the MIB-producing cyanobacterium *Oscillatoria perornata* from a west Mississippi catfish pond and the MIB-producing *Pseudanabaena* sp. (strain LW397) from Lake Whitehurst, Virginia, used as a city water supply reservoir. The cyanobacterium *Oscillatoria agardhii*, not a MIB-producer, and the green alga *Selenastrum capricornutum*, found in catfish ponds in the southeastern United States, were included in the bioassay to help determine potential broad-spectrum toxicity of the commercial products.

MATERIALS AND METHODS

An isolate of *O. perornata* was obtained from a water sample collected from a west Mississippi catfish pond (van der Ploeg et al. 1995). A culture of *Pseudanabaena* sp. (strain LW 397) was obtained from George Izaguirre, Metropolitan Water District of Southern California, La Verne, California. An isolate of *O. agardhii* was also obtained from a west Mississippi catfish pond and *S. capricornutum* was obtained from Dr. J. C. Greene, United States Environmental Protection Agency, Corvallis, Oregon. Each culture was maintained separately in continuous, steady-state growth using the conditions outlined in Schrader et al. (1997) to provide a source of cells growing at a fairly constant rate.

The rapid bioassay of Schrader et al. (1997) was used to evaluate the commercial products. The following commercial products were evaluated in this study: 1) AlgaeFix®[2]; 2) MICROBE-LIFT® Barley Straw Concentrated Extract[3]; 3) SAVIO Natural Barley Extract™[4]; and 4) ZeroTol™[5]. Stock solutions of each commercial product were made in sterile,

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[2]AlgaeFix® is a registered trademark of Aquarium Pharmaceuticals, Inc., Chalfont, PA.

[3]MICROBE-LIFT® Barley Straw Concentrated Extract is a registered trademark of Ecological Laboratories, Inc., Freeport, NY.

[4]SAVIO Natural Barley Extract™ is a trademark of SAVIO Engineering, Inc., Sante Fe, NM.

[5]ZeroTol™ is a trademark of BioSafe Systems, Glastonbury, CT.

deionized water [0.00004, 0.0004, 0.004, 0.04, and 0.4% (v/v) for AlgaeFix and ZeroTol; 0.01, 0.1, 1.0, and 10.0% (v/v) for each barley straw extract product]. Stock solutions of AlgaeFix and ZeroTol were added (50 µL per well) to appropriate microplate wells (96-well polystyrene microplate, Corning Incorporated, Corning, NY) containing 150 µL of culture from one of the continuous cultures. Stock solutions and undiluted solutions of MICROBE-LIFT barley straw extract and SAVIO barley extract were added (10 µL per well) to appropriate wells containing 190 µL of culture from one of the continuous cultures. Final treatment concentrations were 0.00001, 0.0001, 0.001, 0.01, and 0.1% (v/v) for AlgaeFix and ZeroTol while final treatment concentrations for MICROBE-LIFT barley straw extract and SAVIO barley extract were 0.0005, 0.005, 0.05, 0.5, and 5.0% (v/v). Sterile, deionized water was added to the controls. Three replications were used for each commercial product concentration and control, and experiments were repeated. Microplates were placed in a growth chamber held at 29 ± 1°C and were illuminated continuously by fluorescent lights (40 W, cool white) at a photon flux density of 21 to 27 $\mu E/m^2/s$. Absorbance measurements of each well were measured at 650 nm at 24-h intervals for 4 days using a Packard model SpectraCount microplate photometer (Packard Instrument Company, Meriden, CT). Mean values and standard deviations of absorbance measurements were calculated and graphed to determine the lowest-observed-effect concentration (LOEC), lowest-complete-inhibition concentration (LCIC), and 96-h IC50 (50% inhibition concentration).

AlgaeFix, marketed as a liquid formulation, contains 4.5% of the active ingredient poly[oxyethylene-(dimethyliminio) ethylene(dimethyliminio)ethylene dichloride], and product information states that AlgaeFix can cause the cellular membranes of algae to leak and may also adversely affect nutrient and ion flow across cellular membranes. This product is registered with the USEPA for use in fish aquaria and ornamental water garden ponds.

ZeroTol is also registered with the USEPA for use as a fungicide and algicide in greenhouses, nurseries, and garden centers, and it contains 27% of the active ingredient hydrogen dioxide. Hydrogen dioxide is derived by combining hydrogen peroxide with peracetic acid. Previous research found that hydrogen peroxide has algicidal activity (Kay et al. 1982); however, hydrogen peroxide can quickly break down when exposed to sunlight. According to product information, ZeroTol is stabilized to help prevent rapid breakdown.

Both barley straw extract products contain material from decomposed barley straw. None of the products evaluated in this study have been label-approved by the USEPA for use in catfish aquaculture. The IC50 values for AlgaeFix and ZeroTol are based upon active ingredients while LOEC and LCIC values are based upon product formulations.

RESULTS AND DISCUSSION

Research dealing with the discovery of natural and natural-based algicides has garnered more attention recently due to increased environmental concerns about and the negative attributes of currently available algicides for controlling noxious cyanobacteria in freshwater ecosystems. There have been several different approaches and bioassay methods used in the discovery of novel natural-based algicides. Gross et al. (1991) used an agar-overlay plate method to discover the allelochemical fischerellin that is produced by the cyanobacterium *Fischerella muscicola* and inhibits the growth of other species of cyanobacteria and certain species of green algae (chlorophytes). More recently, Gross et al. (1996) have used a bioassay in which the inhibition of alkaline phosphatase activity is measured by fluorescence spectrometry to identify algicidal polyphenols produced by the aquatic plant *Myriophyllum spicatum*. Walker and Higginbotham (2000) used shake-flask culture studies to help discover an aquatic bacterium (SG-3) that lyses cyanobacteria including *O. perornata*. Scale-up studies were later performed using 757-L polypropylene tanks containing catfish pond water with blooms of *Oscillatoria* spp. (e.g., *O. perornata*) to further evaluate the algicidal properties of bacterium SG-3 (Walker 2003).

The rapid bioassay of Schrader et al. (1997) has been found to be reproducible and reliable as a primary evaluation of compounds and commercial products for algicidal selectivity. It does not provide definitive information as to the efficacy of certain compounds and commercial products in aquatic environments. However, a compound or commercial product that does not show promise in the primary evaluation (i.e., bioassay) is unlikely to be effective when tested in secondary or scale-up type studies such as the use of limnocorrals (fiberglass enclosures) placed in catfish aquaculture ponds for efficacy testing of compounds and commercial products (see Schrader et al. 2000).

AlgaeFix was effective at 0.01% (v/v) (or 4.5 mg/L of the antimicrobial active ingredient) against each of the test organisms based upon LCIC results. The LOEC and LCIC values are above the label-recommended initial application rate of 0.0026% (v/v) (or 1.2 mg/L of the active ingredient), and AlgaeFix was not found to be selectively toxic towards the cyanobacteria tested when compared to *S. capricornutum* based upon IC50 results. The IC50 values were all higher for each cyanobacterium tested (1.8-2.8 mg/L) compared to an IC50 of 0.9 mg/L for *S. capricornutum* (Table 1). Also, AlgaeFix is not economically practical for use in large commercial-size catfish ponds when compared to diuron label application rates (10 µg/L) (Tucker and Leard 1999) and the inexpensive commercial price of $13/kg for diuron.

Results from this study showed that neither of the barley extract products tested was effective in killing the four test organisms (Table 1). The initial label-recommended application rate of MICROBE-LIFT and SAVIO Barley Extract are 0.0066% (v/v) and 0.0078% (v/v), respectively. The LCIC and LOEC for both products was >5% (the highest concentration tested was 5%). The 96-h IC50 was not determined for these two barley extract products.

Previous research by Newman and Barrett (1993) demonstrated that rotting barley straw inhibited the growth of the cyanobacterium *Microcystis aeruginosa*. However, research by Wills et al. (1999) found that decomposing barley straw when placed in Mississippi catfish ponds did not reduce the occurrence of musty off-flavor in catfish. The lack of toxicity of the barley extracts towards the cyanobacteria tested in this study is unclear. Pillinger et al. (1994) suggest that the oxidation of phenolic compounds and lignin derivatives from decomposing barley straw under aerobic conditions may yield quinones. Several

TABLE 1. RAPID SCREENING RESULTS OF COMMERCIAL PRODUCTS TO EVALUATE TOXICITY TOWARDS SELECTED PHYTOPLANKTON. PERCENTAGES ARE BASED UPON A VOLUME/VOLUME BASIS.

	Test Organism											
	O. perornata			*O. agardhii*			*Pseudanabaena* sp. LW397			*S. capricornutum*		
Test Product	LOEC	LCIC	IC50	LOEC	LCIC	IC50	LOEC	LCIC	IC50	LOEC	LCIC	IC50
AlgaeFix	0.01%	0.01%	1.8	0.01%	0.01%	2.8	0.01%	0.01%	2.8	0.01%	0.01%	0.9
MICROBE-LIFT Barley Extract	>5.0%	>5.0%	ND	>5.0%	>5.0%	ND	>5.0%	>5.0%	ND	>5.0%	>5.0%	ND
SAVIO Barley Extract	>5.0%	>5.0%	ND	>5.0%	>5.0%	ND	>5.0%	>5.0%	ND	>5.0%	>5.0%	ND
ZeroTol	0.0001%	0.001%	0.4	0.001%	0.001%	1.4	0.0001%	0.001%	1.7	0.001%	0.01%	3.4

LOEC = Lowest-observed-effect concentration; LCIC = Lowest-complete-inhibition concentration; IC50 = 50% Inhibition concentration after 96 hours and expressed as mg/L of active ingredient; ND = Not determined.

quinones have been found to be selectively toxic towards *O. perornata* in laboratory and pond efficacy studies (Schrader et al. 1998, Schrader et al. 2003). However, the chemical compositions of the two barley extracts were not elucidated in this study to determine the presence or types of quinones.

ZeroTol was the most selectively toxic of the four commercial products evaluated, with a LCIC of 0.001% for each cyanobacterium tested compared to a LCIC of 0.01% for *S. capricornutum* (Table 1). The IC50 values of ZeroTol for *O. perornata* and *Pseudanabaena* sp. LW397 were determined to be 0.4 and 1.7 mg/L, respectively, compared to an IC50 of 3.4 mg/L for *S. capricornutum*. Although diuron is more toxic towards *O. perornata*, ZeroTol can be considered more environmentally safe due to low environmental persistence and its degradation products of water and oxygen.

ZeroTol appears to be the most promising of the commercial products evaluated in this study for potential use in managing noxious types of cyanobacteria in catfish aquaculture ponds. Efficacy studies need to be performed in a dose-response format in catfish ponds using limnocorrals (fiberglass enclosures) (Schrader et al. 2000) to help further evaluate the effects of ZeroTol on phytoplankton community structure. In addition, studies need to be conducted to evaluate the toxicity of ZeroTol towards channel catfish. Such studies would help determine if there is a sufficient margin between phytotoxic and ichthyotoxic concentrations of ZeroTol for its use in catfish aquaculture.

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LITERATURE CITED

Gross, E. M., C. P. Wolk and F. Jüttner. 1991. Fischerellin, a new allelochemical from the freshwater cyanobacterium *Fischerella muscicola*. J. Phycol. 27:686-692.

Gross, E. M., H. Meyer and G. Schilling. Release and ecological impact of algicidal hydrolyzable polyphenols in *Myriophyllum spicatum*. Phytochemistry 41:133-138.

Kay, S. H., P. C. Quimby, Jr. and J. D. Ouzts. 1982. H_2O_2: a potential algicide for aquaculture. Proc. So. Weed Sci. Soc. 35:275-289.

Newman, J. R. and P. R. F. Barrett. 1993. Control of *Microcystis aeruginosa* by decomposing barley straw. J. Aquat. Plant Manage. 31:203-206.

Peterson, H. G., S. E. Hrudey, I. A. Cantin, T. R. Perley and S. L. Kenefick. 1995. Physiological toxicity, cell membrane damage, and the release of organic carbon and geosmin by *Aphanizomenon flos-aquae* after exposure to water treatment chemicals. Water Res. 29:1515-1523.

Pillinger, J. M., J. A. Cooper and I. Ridge. 1994. Role of phenolic compounds in the antialgal activity of barley straw. J. Chem. Ecol. 20:1557-1569.

Schrader, K. K., M. Q. de Regt, C. S. Tucker and S. O. Duke. 1997. A rapid bioassay for selective algicides. Weed Technol. 11:767-774.

Schrader, K. K., M. Q. de Regt, P. R. Tidwell, C. S. Tucker and S. O. Duke. 1998. Selective growth inhibition of the musty-odor producing cyanobacterium *Oscillatoria* cf. *chalybea* by natural compounds. Bull. Environ. Contam. Toxicol. 60:651-658.

Schrader, K. K., C. S. Tucker, M. Q. de Regt and S. K. Kingsbury. 2000. Evaluation of limnocorrals for studying the effects of phytotoxic compounds on plankton and water chemistry in aquaculture ponds. J. World Aquacult. Soc. 31:403-415.

Schrader, K. K., A. M. Rimando and S. O. Duke. 2002. Natural compounds for the management of undesirable freshwater phytoplankton blooms, pp. 351-389. *In*: Atta-ur-Rahman (ed.). Studies in natural products chemistry, Vol. 26. Elsevier Science B.V., Amsterdam.

Schrader, K. K., N. P. D. Nanayakkara, C. S. Tucker, A. M. Rimando, M. Ganzera and B. T. Schaneberg. 2003. Novel derivatives of 9,10-anthraquinone are selective algicides against the musty-odor cyanobacterium *Oscillatoria perornata*. Appl. Environ. Microbiol. 69:5319-5327.

Tucker, C. S. and A. T. Leard. 1999. Managing catfish off-flavors with diuron. Fact Sheet 003 (Revised), Thad Cochran National Warmwater Aquaculture Center, Mississippi State University, Stoneville, MS.

van der Ploeg, M., M. E. Dennis and M. Q. de Regt. 1995. Biology of *Oscillatoria* cf. *chalybea*, a 2-methylisoborneol producing blue-green alga of Mississippi catfish ponds. Water Sci. Technol. 31:173-180.

Walker, H. L. and L. R. Higginbotham. 2000. An aquatic bacterium that lyses cyanobacteria associated with off-flavor of channel catfish (*Ictalurus punctatus*). Biol. Control 18:71-78.

Walker, H. L. 2003. Microbial algicides: potential for management of cyanobacteria that cause off-flavor in aquaculture, pp. 147-165. *In*: A. M. Rimando and K. K. Schrader (eds.). Off-flavors in aquaculture. American Chemical Society, Washington, DC.

Wills, G. D., C. S. Tucker and E. J. Jones. 1999. Effect of barley straw for the control of off-flavor in pond-raised catfish. Proc. Sou. Weed Sci. Soc. 52:227-230.