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Acute Toxicity of Saponified Castor Oil to Channel Catfish, *Ictalurus punctatus*, under Laboratory and Field Conditions

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Solricin 135® (Caschem Inc., Bayonne, NJ) has been proposed as an algicide for ponds used to commercially culture channel catfish Ictalurus punctatus. Solricin 135® is an aqueous solution of potassium hydroxide-saponified castor oil containing about 32% (by weight) of the potassium salts of fatty acids and 3% glycerol. Castor oil is a triacylglycerol and about 90% of the fatty acid content is ricinoleic acid (12-hydroxyoleic acid). Ricinoleic acid structurally is similar to naturally occurring alleopathic compounds isolated from Eleocharis microcarpa and other aquatic angiosperms. These compounds are oxygenated, unsaturated fatty acids and, in laboratory bioassays, they inhibit the growth of blue-green algae (Cyanobacteria) to a greater extent than other taxa of algae (van Aller and Pessoney 1982; van Aller et al. 1985). In limited field tests (Pessoney et al. 1984), saponified castor oil showed promise as a relatively selective blue-green algicide at concentrations of 1 to 5 mg/L.

There are no data available describing the toxicity of saponified castor oil to fish. We report here the acute toxicity of Solricin 135® to channel catfish fingerlings under laboratory conditions. Because the solubility of most saponified fatty acids is influenced by the concentration of divalent cations in the water, toxicity was determined at four environmental calcium concentrations. A field test was also conducted to compare the laboratory-derived data to the toxicity of Solricin 135® under conditions typical of commercial channel catfish production ponds.

MATERIALS AND METHODS

Channel catfish fingerlings (mean 13.4 g; SE 0.6 g) were obtained from a nursery pond and held in a 500-L flow-through tank. Fish were fed to satiation every second day using a commercial, 32% crude protein, pelleted feed. Fish were not fed for 48 h before being moved to aquaria.

Four tests were conducted, one test each at calcium concentrations of <0.1, 20, 40, and 80 mg Ca/L. The water used in the tests was groundwater with the following selected chemical characteristics:

calcium, <0.1 mg/L; magnesium, <0.1 mg/L; sodium, 90 mg/L; chloride, 20 mg/L; total alkalinity, 3.9 mEq; pH, 8.3. Reagent grade $CaCl_2 \cdot 2H_2O$ was added to the aquaria to give the desired calcium concentrations. Appropriate quantities of reagent grade NaCl were added to aquaria at the three lowest calcium concentrations to equalize the osmolarity of all the waters used. Tests were conducted under static conditions at 25 ± 2°C in O_2 saturated water. Each test consisted of six treatments: a control and five concentrations of Solricin 135® in a geometric progression of concentrations. Three 65-L aquaria, each containing six fish, were allocated to each treatment. Fish were held in aquaria 24h before addition of Solricin 135®. The tests were terminated after 96 h of exposure. Lethal concentrations, as 96-h LC-01, LC-50, and LC-99, were calculated using the Statistical Analysis System probit procedure (SAS 1982). These values represent theoretical concentrations that kill 1, 50, and 99%, respectively, of the fish within 96 h under the conditions of the test. Solricin 135® contains 35% solids by weight and all concentrations are expressed on a mg/L of solids basis.

Six ponds on the Delta Branch Experiment Station, Stoneville, MS, were used in the field test. The surface area of the ponds was approximately 0.05 ha with an average depth of about 0.75 m. Actual pond volumes were estimated by the chloride-dilution method (Boyd 1979). The ponds are contained within earthen levees and filled with groundwater. Groundwater was added periodically to replace evaporation and seepage losses. Total hardness and alkalinity of pond waters ranged from 3 to 5 mEq. Ponds were stocked in April 1984 with 10,850 adult channel catfish per ha (mean 200 g per fish). Fish were fed daily with a commercial 32% crude protein ration at 1 to 2% of body weight adjusted periodically for weight gain. Throughout the summer, dissolved oxygen concentrations were determined daily in each pond at dawn and dusk and at intervals throughout the night. If dissolved oxygen concentrations fell below 2 mg/L, emergency aeration was provided by a 0.25-kW surface aerator.

During the mid-morning on October 8, 1985, ponds were treated with Solricin 135® at 0, 5, 8, 14, 17, or 24 mg/L. The material was diluted with pond water and evenly splashed over the pond surfaces. At the time of treatment, the pond water temperatures were about 20°C, calcium concentrations ranged from 70 to 80 mg Ca/L, and fish weighed about 0.9 kg each. Five fish were seined from each pond 4, 48, and 168 h after treatment for necropsy and evaluation of gross pathology.

RESULTS AND DISCUSSION

The 96-h LC-50 values in laboratory tests ranged from 25 to 59 mg/L saponified castor oil. The toxicity of saponified castor oil decreased as the calcium concentration in the test water increased (Figure 1). This is the result of the formation of relatively insoluble calcium salts of the fatty acids as environmental calcium concentrations increase. In water of low calcium

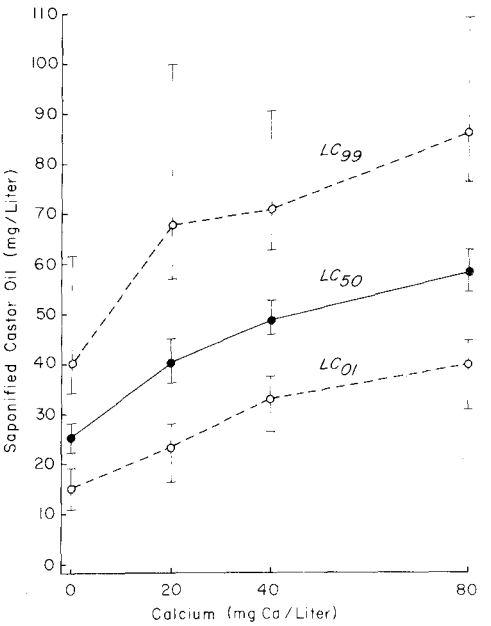

Figure 1. Toxicity of saponified castor oil (Solricin 135®) to channel catfish after 96 h of exposure at four environmental calcium concentrations. Vertical bars represent 95% confidence intervals.

concentration, the toxicity of saponified castor oil was similar to the toxicity of three packaged, commercial soaps to fathead minnows, Pimephales promelas (Henderson et al. 1959). The commercial soaps were primarily mixtures of the sodium salts of stearic, palmitic, and oleic acids. For fathead minnows, the 96-h LC-50 values for the commercial soaps ranged from 29 to 42 mg/L in water with about 10 mg/L Ca. However, in water with about 160 mg/L Ca, Henderson et al. (1959) report 96-h LC-50 values of 1100 to 1800 mg/L for the commercial soaps. Although we did not use water with a calcium concentration this high, it nevertheless appears that saponified castor oil is more toxic to fish than other soaps in waters of moderate to high calcium concentrations. Ricinoleic acid, the predominant fatty acid in castor oil, is hydroxylated at the number 12 carbon and this may render the calcium salts of this fatty acid somewhat more soluble in water than the calcium salts of non-hydroxylated fatty acids.

In all the laboratory tests, most of the fish died within the first 24 h of exposure; no fish died 48 to 96 h after exposure. For example, at the highest calcium concentration (80 mg Ca/L), the 24-, 48-, 72-, and 96-h LC-50 values were 64, 59, 59, and 59 mg/L, respectively. This finding is similar to that of Henderson

Table 1. Cumulative percentage mortality of channel catfish after treatment of ponds with saponified castor oil (Solricin 135®).

Time after treatment	Treatment rate (mg/L)					
	0	5	8	14	17	24
24 h	0	0	0	0	8	20
48 h	0	0	0	0	58	83
72 h	0	0	0	1	74	100
96 h	0	0	0	1	84	100
120 h	0	0	0	1	85	100
144 h	0	0	0	1	86	100

et al. (1959) who found little increase in mortality of fathead minnows after the initial 24 h of exposure to three different commercial soaps.

Fish exposed to toxic concentrations of saponified castor oil in aquaria showed a characteristic behavior pattern. Immediately after the saponified castor oil was added, the fish became hyperactive, increased their ventilation rate, and exhibited a gulping reflex. Within two hours, some fish lost equilibrium or assumed a tail-down position at the water surface. Depending on the treatment rate, some fish recovered from this point. Lethargy, with a periodic gulping reflex, preceded death.

Results of the tests in ponds are not directly comparable to the laboratory data because of the differences in fish sizes, test temperatures, and other factors. However, saponified castor oil appeared to be much more toxic to fish in ponds (Table 1) than in laboratory tests. The concentration of saponified castor oil in the pond where all fish eventually died (24 mg/L) was much less than the 96-h LC-01 (about 40 mg/L) under laboratory conditions at similar environmental calcium concentrations (about 80 mg Ca/L).

The initial behavior of fish in ponds treated with saponified castor oil was similar to that observed in aquaria. Within 2 h of treatment, several distressed fish were observed in the ponds treated with 24 mg/L and 17 mg/L saponified castor oil. These fish were lethargic and swimming slowly in a tail-down position at the surface. Fish were responsive to stimuli and would startle when approached. After 4 h of exposure, gross clinical signs in fish from the pond treated with 24 mg/L saponified castor oil included echymosis of the fins and rectum; petechiation of the head, mouth, and lateral body wall; and rectal prolapse. Copius amounts of viscous brown mucus were noted on the dorsal body wall accompanied by some epidermal depigmentation. Gills were pale with vertical white striations. Internal abnormalities were suggestive of intestinal hypermotility and findings included intussusception, fluid distention of the intestine, and lack of food or fecal matter in the gut. Ricinoleic acid is a potent irritant carthartic when ingested and this may explain the intestinal abnormalities. The liver was pale and mottled, and the

spleen congested. Gross lesions of fish from ponds treated with 17 mg/L, 14 mg/L, and 8 mg/L of saponified castor oil were limited to rectal hemorrhage. Intussusception was noted in fish from ponds treated with 14 mg/L and 5 mg/L. No other abnormality was noted in the fish from the pond treated with 5 mg/L and no abnormalities were noted in fish from the untreated control pond.

By 48-h post-treatment, all fish had died in the pond treated with 24 mg/L saponified castor oil and deterioration of physical condition was obvious in the fish sampled from ponds treated with 17 mg/L and, to a lesser extent, with 14 mg/L saponified castor oil. Fish from ponds treated with 17 mg/L had large areas of depigmentation on the dorsal and lateral surface and were covered with a thick green-brown mucus. Rectal lesions persisted and included hemorrhage, inflammation, and prolapse. The gut was empty except for a thick yellow mucus in the intestine. Intussusception was noted in 2 of 5 fish examined. Abdominal organs were pale and a mild anemia was noted. Dorsal depigmentation and rectal inflammation were also present in fish from ponds treated with 14 and 8 mg/L saponified castor oil. No clinical abnormalities were noted in fish from the untreated control pond or the pond treated with 5 mg/L saponified castor oil.

Fish were again collected from each pond for clinical evaluation 1 week after treatment. In fish examined from the ponds treated with 17, 14, and 8 mg/L saponified castor oil, external lesions were characterized by an abnormally thin mucus layer and rough, dry epidermis. Some dorsal depigmentation was also present in these fish. In 3 of 5 fish examined from the pond treated with 17 mg/L saponified castor oil, the dorsal depigmentation was accompanied by a severe epidermal ulceration that is characteristic of external Flexibacter columnaris infections. However, the presence of this organism was not confirmed. Flexibacter columnaris was identified with light microscopy from scrapings of skin mucus in 1 of 5 fish from the pond treated with 14 mg/L saponified castor oil. Fish from the untreated pond and the pond treated with 5 mg/L saponified castor oil were normal.

The apparent increased toxicity of saponified castor oil under field conditions as compared to laboratory tests is probably the result of the interaction between the direct effects of the toxicant and the effects of concurrent suboptimal environmental factors. One of the major toxic effects of surface-active agents on fish is disturbance of gas-exchange at the gill surface and subsequent hypoxia (Schmid and Mann 1961). In our laboratory tests, dissolved oxygen concentrations were maintained at optimum levels (saturation) throughout the test periods. In commercial catfish ponds, dissolved oxygen concentrations vary diurnally and may be less than 50% of saturation on many summer and fall mornings and may exceed 150% of saturation during the afternoon. Furthermore, the use of algicides usually results in decreased dissolved oxygen concentrations in catfish ponds because of their effect on net photosynthetic oxygen production. For example, in

the pond treated with 24 mg/L saponified castor oil, dissolved oxygen levels at dawn exceeded 40% of saturation during the week prior to treatment. On the three consecutive mornings after treatment, dissolved oxygen levels in this pond were 30%, 22%, and 10% of saturation, respectively. The net effect on fish health of a toxicant that interferes with fish respiration will therefore be greater in ponds than in most laboratory tests because of the suboptimal environmental conditions typical of fish culture ponds. Unfortunately, it will be difficult to accurately predict lethal concentrations in ponds because of great pond-to-pond differences in water quality. The long-term effects of inadvertent over-treatment of ponds with saponified castor oil also will be difficult to quantify because of possible secondary effects on fish health. Sublethal hypoxia and cartharsis in fish exposed to more than about 10 mg/L saponified castor oil may stress the animal and result in infection by opportunistic bacterial pathogens such as Flexibacter columnaris or Aeromonas spp.

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