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ROTENONE TOLERANCE IN MOSQUITOFISH

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ABSTRACT

Resistant mosquitofish showed a 1.8-fold tolerance to rotenone over a susceptible strain. The 24-h LC₅₀ values for rotenone in susceptible and resistant mosquitofish were 0.017 ppm and 0.031 ppm, respectively. Results with sesamex, an inhibitor of mixed-function oxidase (mfo) enzymes, indicated that rotenone tolerance in mosquitofish is solely the result of increased levels of mfo enzymes.

INTRODUCTION

An increasingly important effect of environmental contamination with insecticides, particularly with DDT and allied chemicals, is that pests resistant to these insecticides are selected and the population becomes difficult to control. Vertebrates usually breed too slowly for such resistant populations to be produced, but they do occur. Thus, in 1963, a DDT-resistant population of mosquitofish, *Gambusia affinis*, was discovered in a drainage ditch adjacent to heavily-sprayed cotton fields near Belzoni, Mississippi (Vinson *et al.*, 1963). Subsequent studies showed these mosquitofish to be resistant to a wide variety of organochlorine and organophosphorus insecticides (Culley & Ferguson, 1969).

Resistance to rotenone is very uncommon among arthropods. However, in 1949, a rotenone-resistant population of Mexican bean beetles was found in the northeastern United States (Brown, 1968). We now report an apparent case of rotenone resistance in mosquitofish found at Belzoni, Mississippi. Their susceptibility to rotenone was compared to that of normal fish collected near State College, Mississippi. This project was established to determine the toxicity of rotenone to resistant and susceptible mosquitofish.

MATERIALS AND METHODS

Resistant mosquitofish were collected from Belzoni, Mississippi; susceptible fish were collected near State College, Mississippi.

Rotenone was dissolved in acetone at a 1% concentration and this stock solution was added to water to yield the desired final concentrations (0.008–0.042 ppm). Control groups received an equal volume of solvent alone. All bioassays were conducted in glass aquaria with fifty fish in 20 litres of dechlorinated tap water at each concentration. Mortality was determined 24 h post-treatment.

The detoxication of rotenone in insects and mammals is primarily via oxidation by microsomal mixed-function oxidase (mfo) enzymes. Sesamex, a known inhibitor of mfo enzymes, was used to determine if any differences in toxicity between the two strains of fish were a result of increased levels of mfo enzymes.

Resistant and susceptible fish were exposed to 2 ppm sesamex (diluted from a 1% (v/v) stock solution of sesamex in acetone) for 24 h in glass aquaria. After the sesamex pretreatment, the fish were exposed to 0.0066 and 0.015 ppm rotenone (twelve fish in 6 litres of water in glass aquaria). Mortality was determined 6 and 24 h post-treatment. These bioassays were replicated three times.

RESULTS AND DISCUSSION

Resistant mosquitofish showed a 1.8-fold tolerance to rotenone over the susceptible strain. The 24-h LC_{50} values for rotenone in susceptible and resistant mosquitofish were 0.017 ppm and 0.031 ppm, respectively. The toxicity of rotenone to sesamex-pretreated fish was essentially the same for both strains. Mortality at 0.0066 ppm, 24 h post-treatment, was 44% for both susceptible and resistant fish. At 0.015 ppm, 6 h post-treatment, the observed mortality was 92% for susceptible and 89% for resistant fish. Sesamex alone produced no mortality in the fish. Sesamex pretreatment indicated that rotenone tolerance in mosquitofish is the result of increased levels of mfo enzymes. Similarly, Ludke *et al.* (1972) showed that sesamex prevented aldrin epoxidation and parathion activation in mosquitofish, presumably by binding mfo enzymes.

Resistant mosquitofish from the Belzoni area have had no known exposure to rotenone. High levels of mfo enzymes in resistant fish are apparently not the result of prior exposure to rotenone. This implies that some other factor(s) in the environment caused high levels of mfo enzymes in resistant mosquitofish. Resistant mosquitofish are known to contain organochlorine insecticide residues; organochlorine compounds are known inducers of mfo enzymes in mammals. Thus, it is possible that organochlorine residues induced high levels of mfo enzymes in resistant mosquitofish. This would, in effect, confer a cross-tolerance to rotenone in resistant mosquitofish.

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