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MOLLUSCICIDAL ACTIVITY OF AFLATOXIN

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SUMMARY

The Authors studied the molluscicidal activity of culture medium extracts of *Aspergillus parasiticus*, producer of aflatoxin B-1. This substance caused the death of all snails (*Biomphalaria tenagophila*) when used at the concentration of 0.5 ppm. The Authors point out the necessity of field trials using contaminated groundnut meals and foodstuffs, for evaluating their importance in the elimination of the schistosomiasis vector, as well as to find an economic use for this rejected material.

INTRODUCTION

In schistosomiasis prophylaxis it is very important to break the transmission cycle by destroying the intermediate hosts, probably the most efficient and most easily applied method.

A great number of studies has been carried out with molluscicidal substances, the most important being sodium pentachlorophenolate, triethylmorpholine (Frescon) and chlorosalicylnitroaniline (Bayluscide).

The action of aflatoxin on snails, water plants and fishes was studied with the purpose of finding a new molluscicidal substance. Aflatoxin is a toxic metabolite produced by *Aspergillus flavus* and *Aspergillus parasiticus* having a well defined chemical structure and belonging to the complex furocoumarins. SOINE [6], reviewed the natural coumarins, studying their physiological behaviour and describing also their molluscicidal activities. This review aroused our interest in the study of the biological effects of aflatoxin.

MATERIAL AND METHODS

The adult snails used were of the *Biomphalaria tenagophila* genus, of about 1.5 cm in diameter, bred in laboratories and belonging to a strain obtained in the Capital of the State of São Paulo (Brazil).

To obtain the toxic extract containing aflatoxin, an *Aspergillus parasiticus* strain (strain 15957ii, Tropical Products Institute, England) was cultured in Sabouraud-saccharose medium for 15 days, at room temperature (about 22°C), in Roux flasks. After 15 days the material was filtered through gauze and the toxic principle extracted with 3 portions of 30 ml chloroform each.

The extracts were mixed and concentrated on the water-bath to a volume of approximately 10 ml and the exact volume was made up to 10 ml in volumetric flasks.

For the quantitative determination we used the dilution method, submiting the unknown in thin-layer-chromatography plates (500 mμ thickness) to development with ethyl ether and later with chloroform-methanol (97:3), as described by NABNEY & NESBITT [3]. The

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concentration of the extract was evaluated by comparing the intensity of the fluorescence emitted to that of a standard aflatoxin B-1 solution.

The standard was obtained from Dr. Hesseltine of the Northwestern Regional Research Laboratory, Peoria, Illinois, as crystalline aflatoxin B-1, which was diluted with chloroform to the desired concentrations.

The standardized solution was added to the deionized water (pH 7.0) of the aquariums, to which "Tween 80" was also added as emulsifying agent. A parallel series of tests was performed with drugs considered molluscicidal, such as "Bayluscide 70" (FREITAS & PAULINI [2]), "Frescon" and sodium pentachlorophenolate. As controls, a group of snails was maintained in contact with "Tween 80", another with chloroform and a third group with water only.

Throughout the experimental period no food was supplied and the aquariums were kept in a room maintained at a temperature varying from 22 to 24°C.

To evaluate the molluscicidal activity "*in vitro*" the technique of PESSÔA [4] was used: 1) The product to be tested was diluted to concentrations of 5 ppm or less; 2) Ten snails were used for each test, kept in 500 ml battery jars and distributed into two jars (5 snails in each) with 250 ml of the solution containing the substance to be tested at the various concentrations; 3) The snails were submerged in the solution for 24 hours at laboratory temperature (25-27°C); 4) The snails were removed from the solution, washed under running water, introduced into a new jar containing fresh and aired water and a few leaves of lettuce, and left for another 24 hours; 5) After 24 hours the number of dead snails was determined microscopically.

The fishes used in the tests were of the *Lebistes* genus and the plants those commonly found in aquariums.

RESULTS

Table I shows the molluscicidal effects of aflatoxin in comparison with the other three substances tested and the control groups.

For the fishes, 100% mortality was obtained at a concentration of 10 ppm and 50% mortality at 5 ppm, while all animals survived at the concentrations presenting good molluscicidal activity (0.250 and 0.5 ppm). No toxic effect was observed in the plants at the concentrations studied.

TABLE I

Molluscicidal activity of aflatoxin in comparison with other substances

Product	Concentration (ppm)	Number of snails *	Mortality %
Aflatoxin	0.5	10	100
	0.250	10	90
	0.125	10	40
	0.0625	10	0
Sodium pentachlorophenolate	0.5	10	60
"Frescon"	0.5	10	100
"Bayluscide"	0.5	10	100
Chloroform	0.5	10	0
"Tween 80"	0.5	10	0
Control	—	10	0

* *Biomphalaria tenagophila*

As can be seen in Table I, the concentration of 0.5 ppm was sufficient to eliminate all the snails used in the experiment. This dose is equal in efficacy to the same doses of "Frescon" or "Bayluscide" and superior to the action of the same dose of sodium pentachlorophenolate, which produced a mortality rate of 60%.

Chloroform and "Tween 80", at the concentrations used, did not have a toxic effect on the snails.

A significant difference was found between the toxic doses which produced a 100% mortality to snails and to fishes. Aflatoxin, at a concentration of 0.5 ppm, was sufficient to kill the snails, while it was necessary to use 10 ppm for the fishes. This is very important if we consider the possible use of aflatoxin in breaking the transmission cycle of schistosomiasis by adding it to the water harboring infected snails.

A few comments on the use of aflatoxin in field trials are necessary. First of all, the water treated must be under strict control since it should not be ingested because the significance of aflatoxin to the human organism is still unknown. The economic aspects of *A. flavus* and *A. parasiticus* must also be considered. Such organisms are producers of aflatoxin in foodstuffs and groundnut meals (peanuts mainly), as well as in some other foods like wheat, flour of toasted bread and misso (PURCHIO [5]). In 1962 Brazil exported 21,192 tons of peanuts and 83,677 tons of oil, cakes and flour. This export has fallen in 1966 to 18,437 tons of peanuts and 5,799 tons of oil, cake and flour. According to FONSECA [1] this fall in peanut export is due to aflatoxin, since the importing countries fear the pathological effects of this factor present at times in peanuts. Thus, the drop in export signifies a serious economic loss not only to Brazil, but to all peanut-growing countries. A field trial with aflatoxin would, therefore, be of double value: its possible use in eliminating the infectious snails and the possibility of using the contaminated foodstuffs. We believe that both factors would be important aids to the national health and economy.

RESUMO

Atividade moluscicida da aflatoxina

Os Autores estudaram a atividade moluscicida de extratos de meios de cultivo de *Aspergillus parasiticus*, produtor de aflatoxina B-1. Esta substância, na concentração de 0,5 ppm, determinou a morte de todos os caramujos (*Biomphalaria tenagophila*) experimentados. Os Autores salientam a necessidade da utilização em testes de campo de tortas e rações contaminadas pela micotoxina a fim de avaliar sua importância na eliminação do vector da esquistossomose como, também, encontrar utilidade para aquêle material rejeitado.

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