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Mycotoxins Produced by Fungi Isolated from Wine Cork Stoppers

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Abstract: The research work was conducted to determine the production of mycotoxins from the fungi isolated from wine corks stoppers. When comparing the four fractions of *Alternaria alternata* obtained by the thin layer chromatography with the standards of tenuazonic acid, alter toxin I, altenuene, alternariol, alternariol monomethyl ether and ten toxin, it can be observed that *Alternaria alternata* only produces alter toxin I, altenuene and alternariol monomethyl ether. The results showed that the production of citrinin by *Penicillium citrinum* and of fumonisin B_1 by *Fusarium moniliforme*, due to the fact that the fractions isolated by TLC of these fungi coincide with the controls for such mycotoxins. *Fusarium solani* did not produce fumosin B_1.

Key words: Mycotoxins, cork stopper, *Alternaria alternata*

Introduction

Mycotoxins are a group of secondary metabolites produced by filamentous fungi which may contaminate food, feeds or the raw materials used to produce them. They also produce mycotoxicoses in humans and animals (Moss, 1994).

The genera of mycotoxigenic fungi are mainly represented by *Aspergillus*, *Penicillium* and *Fusarium,* but *Trichoderma*, *Trichothecium* and *Alternaria* are also important as food contaminants or pathogens for plants, among others (Smith, 1983).

Among the diversity of mycotoxins described so far, we will only mention those directly related to the genera that are the objectives of this study.

Citrinin is produced by *Penicillium citrinum*, although it may also be produced by *Penicillium expansum* and *Penicillium verrucosum* and some species of *Aspergillus.* It is a quinone methide with a powerful antibacterial effect, but toxic to humans and animals. It is also a contaminant in cereals such as wheat, maize, barley and oats (Montani *et al.*, 1988; Franco *et al.*, 1996).

Fumonisins are another important group of mycotoxins produced by *Fusarium,* particularly by *Fusarium moniliforme*. The most abundant in nature is fumonisin B_1, which may be related to esophageal cancer in humans. Its hepatotoxic and hepatocancerogenic properties have also been proven. They have also been shown to be nephrotoxic, immunodepressant and embryo toxic for experimental animals (Voss *et al.*, 1990; Nair, 1998; Sweeney and Dobson, 1999). These mycotoxins are contaminants of natural or processed maize (flour) used as animal or human food (Dombrink-Kurtzman and Dvorak, 1999).

Alternaria alternata produces mycotoxin in grain, rice and maize (Ramm *et al.*, 1994; Stack and Prival, 1986). Contamination with grain that has metabolites from the *Alternaria* species may be related to the occurrence of esophageal cancer in some geographical regions (Davis and Stack, 1991). Mycotoxins produced by *Alternaria* and specifically by *Alternaria alternata* are numerous. Among them the most widely studied are: tenuazonic acid (TA), alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), alter toxin I and ten toxin (Griffin and Chu, 1983; Orvehed *et al.*, 1988).

The main goal of the present study is to determine the production of mycotoxins from the fungi previously mentioned, which have been isolated from wine corks.

Materials and Methods

Fungi isolated from wine corks stoppers, those which according to the corresponding literature were capable of elaborating and accumulating mycotoxins, used in this study. These fungi were: *Alternaria alternata*, *Penicillium citrinum*, *Fusarium moniliforme* and *Fusarium solani.*

The microorganisms already mentioned were cultivated in Petri dishes with 2% malt extract agar (MEA) at a temperature of 28 °C during 7 days. From these, discs of 6 mm diameter were obtained. These discs were placed on Merck® chromatoplates of silicagel at a distance of 2 cm from the lower edge for one minute. The chromatoplates had previously been activated at a temperature of 105 °C for 90 min.

In the same way, 10 µl of the specific mycotoxin standards for each microorganism, prepared in a concentration of 25 µl of the specific mycotoxin standards for each microorganism, prepared in a concentration of 25 µl/ml of chloroform, were placed next to each disc.

The mycotoxins assayed were: tenuazonic acid (Sigma®), ten toxin (Sigma®), Alternariol (Sigma®), altenuene (Sigma®), alternariol monomethyl ether (Sigma®), alter toxin I (Sigma®), citrinin (Sigma®) and fumonisin B_1 (Sigma®).

Thin layer chromatography (TLC) was carried out using of benzene/methanol/glacial (96:6:2, v:v:v) to make it

Table 1: Values of Rf of the mycotoxins produced by *Alternaria alternata*

Mycotoxin	Rf	
	Standard	*Alternaria alternata*
Tenuazonic acid (TA)	0.06	ND
Alter toxin I (ATX-I)	0.26	0.26
Altenuene (ALT)	0.13	0.13
Alternariol (AOH)	0.18	0.18
Alternariol monomethyl ether (AME)	0.38	0.38
Tentoxin (TEN)	0.15	ND

Table 2: Values of the Rf of the mycotoxins produced by *Penicillium citrinum*, *Fusarium moniliforme* and *Fusarium solani*

Microorganism	Mycotoxin	Rf	
		Standard	Microorganism
Penicillium citrinum	Citrinin	0.75	0.75
Fusarium moniliforme	Fumonisin B_1	0.71	0.71
Fusarium solanum	Fumonisin B_1	0.71	ND

possible to detect the mycotoxins produced by *Alternaria alternata* and a mixture of chloroform/methanol (50:50, v:v) to detect the mycotoxins produced by *Penicillium citrinum*, *Fusarium moniliforme* and *Fusarium solani*. The chromato-plates removed after 90 minutes, or when the solvent reached 4 cm from the upper edge of the chromatoplates. They were dried mechanically.

Reading the chromato-plates was carried out by observing the chromatographic spots from the discs containing the microorganisms and their standards was carried out under ultraviolet rays with λ of 254 and 356 nanometers.

Results and Discussion

Fig. 1, illustrates the results obtained from the study of the production of mycotoxins by *Alternaria alternata* after TLC. Fig. 2 shows the results of the production of citrinin by *Penicillium citrinum* and fumonisin B_1 by *Fusarium moniliforme* and *Fusarium solani*.

Table 1 shows the values of the Rf corresponding to the chromatographic spots of the mycotoxins produced by *Alternaria alternata*. Table 2 shows the values of the Rf corresponding to the chromatographic spots of the mycotoxins produced by *Penicillium citrinuim*, *Fusarium moniliforme* and *Fusarium solani*.

When comparing the four fractions of *Alternaria alternata* obtained by the thin layer chromatography with the standards of tenuazonic acid, alter toxin I, altenuene, alternariol, alternariol monomethyl ether and ten toxin, it can be observed that *Alternaria alternata* only produces alter toxin I, altenuene and alternariol monomethyl ether. *Alternaria alternata* produce neither tenuazonic acid nor ten toxin under the experimental condition of this study.

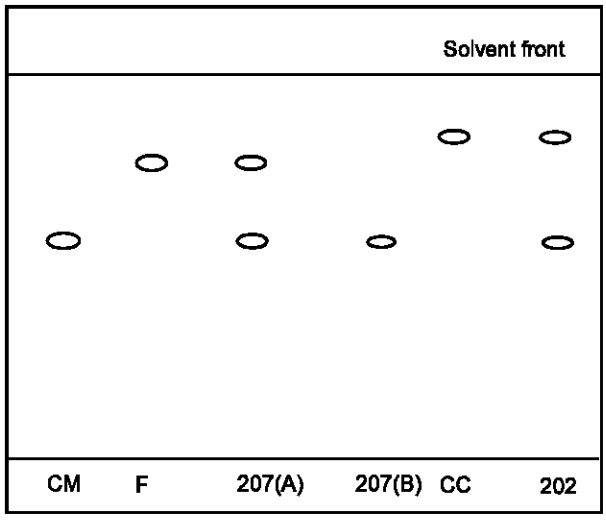

Fig. 1: TLC of the mycotoxins produced by *Alternaria alternata* 201: *Alternaria alternata*, TA: Tenuazoic acid, ATX-I: Alter toxin I, ALT: Altenuene, AOH: Alternariol, AME: Alternariol monomethyl ether, TEN, ten toxin

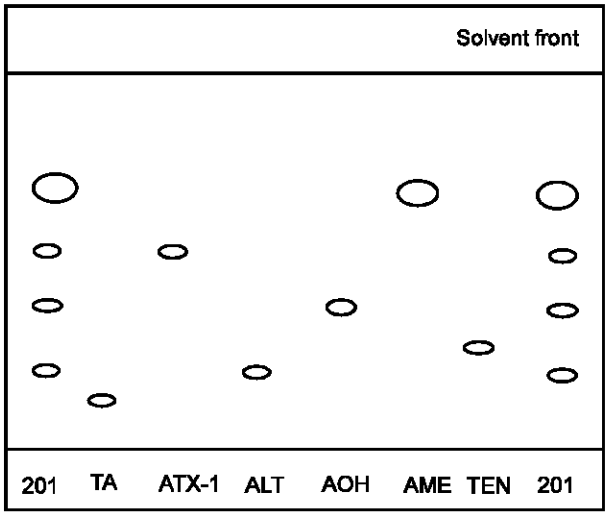

Fig. 2: TLC of the mycotoxins produced by *penicillium citrinum*, *Fusarium moniliforme* and *fusarium solani* CM: Control (2 % malt extract agar), F: Fumonisin, B_1: Control 207(A): *Fusarium moniliforme*, 207(B) : *Fusarium solani*, CC: Citrinin Control, 202: *Penicillium citrinum*

The production of ten toxin is conditioned by a limited quantity of phosphate in the cultivation environment (Ramm *et al.*, 1994), which may have affected the results. Tenuazonic acid is described by Stinson *et al.*, 1980 as a mycotoxin which may be produced by numerous species of *Alternaria*, in cultures isolated from different sources. It

is considered the most important *Alternaria alternata* toxic substance although its production is influenced by nitrogen concentration in a cultivation environment. However, isolated *Alternaria alternata* did not produce tenuazonic acid in this study.

Generally, the production of mycotoxins by *Alternaria alternata* is conditioned by high water activity (a_w), by the incubation temperature, by the pH substrate and by the type of substrate in which the microorganism grows (Burroughs *et al.*, 1976; Magan *et al.*, 1984).

The results obtained illustrate the production of citrinin by *Penicillium citrinum* and of fumonisin B_1 by *Fusarium moniliforme*, due to the fact that the fractions isolated by TLC of these fungi coincide with the controls for such mycotoxins. *Fusarium solani* did not produce fumosin B_1. As with the mycotoxins produced by *Alternaria alternata*, the production of citrinin and fumonisin B_1 depend on water activity (*Fusarium moniliforme* does not produce fumonisins below 0.87 water activity), on environmental, pH, on temperature and on the incubation period of *Penicillium citrinum* and *Fusarium moniliforme* (Montani *et al.*, 1988; Sweeney and Dobson, 1999; Alberts *et al.*, 1990; Wheeler *et al.*, 1991).

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