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Antifungal Activity of Seselin in Protecting Stored Maize from *Aspergillus flavus*

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Abstract: The aim of the present study to evaluate the activity of seselin on *A. flavus*. Seselin, a coumarin, was isolated from hexane extracts of aerial parts of *Decatropis bicolor*. Seselin exhibited fungistatic activity against *Aspergillus flavus* at a concentration of 31.25 μg mL^{-1}. When tested on stored maize var. Cacahuazintle, it was found that seselin, at 1.0 and 2.0 mg of seselin/100 g of grain, offered the same degree of protection against *A. flavus* as that offered by daconil (100 mg) or propionic acid (0.2 mg).

Key words: Seselin, antifungal activity, stored maize, protection

INTRODUCTION

The production of corn silage is essentially based on the principle of preservation under anaerobic conditions together with the growth of lactic acid bacteria, which promote a natural fermentation, lowering the pH to a level at which clostridial growth is inhibited. These conditions are unfavorable for the growth of most fungi. However, poor storage conditions (insufficient drying, condensation, heating, leakage of rainwater or insect infection) could lead to growth of anaerobic and microareobic acid-tolerant fungi. Among of these fungi species of toxigenic fungi as *Aspergillus flavus* Link which produces aflatoxins and they may be the cause of carcinomas mainly in the liver and lungs (Blum, 2002; Van-Vlett *et al*., 2002). Besides the yield of major food including corn nearly 20% by pathogenic fungi (Tarun and Prem, 2006).

Fungicides are one of the main methods used to control pathogenic fungi; but these synthetic compounds may produce resistance plagues and might be carcinogenic and teratogenic (Diop *et al*., 1999). Pesticides derived from plants have been found to be nontoxic with mammals and easily biodegradable (Montes-Belmont, 1996).

Decatropis bicolor (Zucc.) Radlk (Rutaceae) is a small tree, which has been used in folk medicine in Mexico against bacterial infections and as an anti-inflammatory agent (Aguila *et al*., 1994). In earlier study, we found that the hexane extract of *D. bicolor* has antifungal activity on *A. flavus* Link (Cárdenas *et al*., 2003). On the other hand, from the aerial parts of this plant have been reported the presence of alkaloids dictamnine and skimmianine (Dominguez *et al*., 1971) and five coumarins, namely heraclenin, seselin, psoralen, imperatorin and heraclenol, all of which have anti-inflammatory activity (García-Argáez *et al*., 2000). Seselin was also isolated from *Acronychya laurifolia* (Rahmani *et al*., 1996). On the basis of these antecedents, our objective in the present study consisted evaluating the activity of seselin on *A. flavus*.

MATERIALS AND METHODS

Plant material: *D. bicolor* was collected in May 2002 in San Luis Potosí State and identified by José García Pérez, a taxonomist. A voucher specimen (SPLM37574) was deposited with the Isidro Palacios Herbarium of the Universidad Autónoma de San Luis Potosí.

Isolation of seselin: Dried and milled aerial parts (3.0 kg) of the plant were macerated with hexane for 10 days and the solution was evaporated to dryness under reduced pressure. The residue, a brown solid, was analyzed using silica gel chromatography. The column was developed with hexane. The polarity was increased by adding ethyl acetate to provide 24 fractions of 50 mL each. Fractions with identical results from TLC were combined. Seselin was isolated from the combined fractions 5 and 6.

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Table 1: Minimum inhibitory concentration and minimum fungicidal concentration of seselin

	Concentration (μg mL^{-1})								
	6.25	15.6	31.25	46.9	52.5	78.1	93.7	109.3	125
DSB[a]	++	+	-	-	-	-	-	-	-
Czapek agar	++	+	±	±	±	±	±	±	±

[a]Dextrose Sabouraud broth; +, Growth; -, No growth

Minimum Inhibitory Concentration (MIC): MIC was determined by serial dilution (Sidney *et al*., 1978). Tests were performed in dextrose Sabouraud broth. Different concentrations of seselin in DMSO were prepared in 4 mL of broth to obtain final concentrations ranging from 6.25 to 125 μg mL^{-1}. These solutions were placed in culture tubes and the tubes inoculated with *Aspergillus flavus* Link (SRRC 1273 from the National Center for Agricultural Utilization, Peoria IL) at a concentration of ~ 1000 spores per 0.1 mL of suspension. Tubes were incubated at 28±1°C for 72 h. MIC was determined when no growth of the microorganism was evident. The concentrations were increased or decreased as necessary.

Fungicidal or fungistatic activity: Tubes of dextrose Sabouraud broth containing the same concentration of seselin as that at which growth was not detected in the MIC were inoculated with 0.1 mL spore suspension and incubated for 72 h at 28±1°C. After incubation, a 0.1 mL sample was dispersed on each plate of Czapek agar and the plates incubated for 72 h at 28±1°C. The concentration at which growth of *A. flavus* was inhibited in dextrose Sabouraud broth but not on Czapek agar was taken as the fungistatic concentration and that at which neither of the two media supported growth as the Minimum Fungicidal Concentration (MFC).

Protection of stored maize: Jars were filled with 100 g of cleaned grain of Maize var. Cacahuzintle. The relative humidity was maintained at 18%. The samples were treated with 0.1, 0.5, 1.0 and 2.0 mg of seselin in DMSO (20 mg/2 mL DMSO), 100 mg of daconil (75%), or 0.2 mg of propionic acid. The last two compounds served as positive controls (CICOPLAFEST, 1998). Each treatment was replicated four times.

After the treatment, the samples were inoculated with 10 000 spores of *Aspergillus flavus* (1.0 mL of the spore suspension) and incubated at 28°C for 20 days. The humidity was controlled with a saturated solution of KCl and was measured at the beginning and the end of the storage period. After 20 days, the samples were disinfected with 2.0% solution of sodium hypochlorite and ground in a blender. Plates of Czapek agar were inoculated with serial dilutions of ground maize in agar-water (0.12%), incubated at 28°C for 72 h and the number of colonies per g of grain was counted (Cárdenas, 1998).

Table 2: Effect of seselin, daconil and propionic acid in controlling the growth of *A. flavus* Link on grains of maize var. Cacahuzintle stored for 20 days at 28°C

Treatments	mg/100 g ground maize	No. (mean) of spores/g of ground maize
Seselin	2.0	0.0
Seselin	1.0	146.6
Seselin	0.5	156666.7*
Seselin	0.1	416666.7*
Daconil (75% active)	100.0	0.0
Propionic acid	0.2	0.0

Significant difference *p<0.05

The results were expressed as mean values. Statistical significance of the difference among the means of the test and controls studies was established by one-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple-comparison test, using NCSS computer package; p-values <0.05 were considered significant.

RESULTS

The hexane extract of *D. bicolor* was column chromatographed on silica gel. From Fr. 5-6 was obtained a compound which was analyzed by ^{1}HNMR, ^{13}CNMR and IR and was identified as the coumarin seselin which has been previously isolated from *Acronychya laurifolia*.

In earlier study, we found that the hexane extract of *D. bicolor* that MIC and MFC of this extract were the same, namely 12.50 μg mL^{-1}; however, in this study is reported that seselin was fungistatic against *A. flavus* at a concentration of 31.25 μg mL^{-1} in liquid culture (Table 1).

The degree of protection afforded by seselin (1.0 and 2.0 mg) to maize var. Cacahuzintle was similar to that by daconil (100 mg) or propionic acid (0.2 mg) (Table 2).

DISCUSSION

The results observed in Table 1 and that obtained with hexane extract suggest that its major activity in comparison with seselin might be due to the synergistic action of some other antifungal extract components like other as coumarins (García-Argaez *et al*., 2000).

Seselin was found to be highly toxic to eggs of *Tetranychus urticae* (Tanaka *et al*., 1985); also displayed significant antifungal activity against *Cladosporium cladosporioides* (Bandara *et al*., 1991). There are reports that this compound possesses various effects,

moderately cytotoxic (Gumatilaka *et al*., 1994), analgesic and antiinflammatory activities (Tandan *et al*., 1990). *However*, there are no reports of the effect of this compound on *Aspergillus flavus*.

This fungal growth reduces nutritional value and could result in the production of mycotoxins and allergenic spores (Adhikari *et al*., 2004) that constitute a risk factor for human and animal health. For these reasons the need to search for potential antifungal agents from natural sources of non-toxic and easily biodegradable fungicides safe for mammals.

Therefore, seselin could be recommended for protecting grains against *A. flavus* especially if we consider that this compound did not show any acute toxic effect in mice at doses up to 6 g kg^{-1} (Tandan *et al*., 1990).

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