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INHIBITORY EFFECT OF BORIC ACID ON THE DEVELOPMENT AND AFLATOXIN PRODUCTION BY ASPERGILLUS FLAVUS AND ASPERGILLUS PARASITICUS

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SUMMARY

*The Boric acid was tested against **Aspergillus flavus** (NRRL 3251, NRRL5520) and **Aspergillus parasiticus** (UNBF A12, NRRL 2999) at concentrations among an 0,25· 0,5 and 1,0% showing an inhibition over the development of these fungi and resulting on a non aflatoxin production. The results suggest that this additive is efficient to control the fungi of the **A. flavus** group because it promoves the growth inhibition at the highest degree allowed by the Brazilian Law (0,5%).*

RESUMEN

[Efecto inhibitorio del ácido bórico en el desarrollo y la producción de aflotoxinas por Aspergillus flavus y A. parasiticus.]

*El ácido bórico fué analizado en las concentraciones de 0,25%; 0,5% y 1,0%, demostrando su eficacia al inhibir el crecimiento de **Aspergillus flavus** (NRRL 3251 y NRRL 5520) y **Aspergillus parasiticus** (UNBF A12 y NRRL 2999), como también la producción de aflatoxinas. Los resultados sugieren que este aditivo es eficiente en el control de hongos del grupo **A. flavus** inhibiendo el crecimiento de los mismos en la concentración máxima permitida por la legislación brasileña (0,5%).*

INTRODUCTION

A great number of fungi produces toxic metabolites, named mycotoxins that cause serious diseases to man, animals (7). Among all the toxic metabolites, aflatoxins are the highly toxic and carcinogenic toxins that are produced by the fungi of the ***A. flavus*** group mainly ***A. parasiticus*** and ***A. flavus*** that may produce other kinds of mycotoxins too (5).

Efficient measure to control the development of fungi of this group are fundamental to obtain foods and feeds free of contamination for the human and animal consumption.

The Boric acid possesses good preservative properties and may be used only on the cheese conservation (4). The Boric acid and its derivatives act on the enzimes of the phosphoric compound metabolism (6).

This research tries to investigate the effect of this additive on the development and aflatoxin production by *A. **flavus*** and ***A. parasiticus.***

MATERIAL AND METHODS

The fungi utilized belong to the *Aspergillus flavus* group: ***A. flavus*** NRRL 3251 and NRRL 5520 are producers of aflatoxins (AT) B_1 and B_2 ***A. parasiticus*** NRRL 2999 and A12 are both producers of AT B_1, B_2, G_1 and G_2 The samples with NRRL abbreviature are from USDA Northern Regional Research Laboratory, Peoria-Illinois, USA and those with UNBF A12 are from the culture collection of the Phytopathology Laboratory of the University of Brasilia, Brazil.

Culture conservation was carried out in Czapek-agar test tubes (9). Spores of the fungi were inoculated in Petri dishes containing ADM (Aspergillus Differential Medium (1), and incubated for 72 hours at 28°C. Mycelial agar discs of 3 mm in diameter from the ADM were cut of each fungi and inoculated in the center of Petri dishes containing CAM (coconut agar medium) (8) ammended with 0,25, 0,5, 1,0% concentration of Boric acid diluted in water.

The CAM inoculated Petri dishes were incubated for 5 days at 28°C. Three CAM Petri dishes were inoculated to each additive concentration and CAM without the additive was used to control all the fungi. After 5 days of incubation, both colonies pigmentation and diameter and the degree of sporulation as well as fluorescence intensity and its color were observed under the long wave of ultra violet light. A number of 0 to 4 was used to evaluate the sporulation degree. This estimate was used to compare colonies that grew on CAM without the additive

To observe the effect of the acidity CAM ammended with 20% HCL. These plates were inoculated with the same fungi, to simulate the pH of the medium with the acid additive.

Experiments with the liquid medium YES (2) ammended with Boric acid were run. Spores of the fungi from Czapek agar cultures having a 7 to 15 days period were used as inoculum, this is the best period of the spore age for the aflatoxin production (3). The spores were collected with a 0,01% tween 80 solution after shaking and 10^6 Cfu/ml was used as a Standard inoculum for all the fungi. One ml (10^6 CFU/ml) of each spore suspension of each fungus was inoculated into Erlenmayers flask containing 50 ml of YES with the different additive concentrations separately, and were incubated in a stationary culture for 14 days at 28°C. After the incubation period the mycelial mass was filtered on cheese cloth to determine the dry weight. To each filtrate of each fungus and concentration, 2 volumes of chloroform were added and shaked 30 times to complete aflatoxin extraction. The chloroform fraction was collected and the aflatoxin estimate was carried out by Spectrophotometric technique (Beckman DNG) using the value of 1 DO at 360 nm to 15 ug/ml of aflatoxins.

RESULT AND DISCUSSION

Fungi were able to grow on CAM, only with of Boric acid. The colonies diameter, in this con tion, was smaller when compared with the contr orange pigment (OP) was visible to the NRRL Sporulation was inhibited in all the fungi samples 1). On acidified CAM tests, fungi grew up and pr OP and AT similar to the controls. Sporulation deg not changed except for ***A. parasiticus*** UNBF A12, has shown less sporulation (Table 2).

In liquid medium (YES), the results were sim the ones observed in solid medium (CAM), the gro the four fungi samples tested with the 0,5% a concentration not being observed.

The AT production and mycelium dry weight ally decrease with the rise of acid concentration (Ta

The results are very significant because inh occurred at the maximum allowed acid concen (0,5%), then the so called inhibition was not due promoted acidity by the additive, because in ac CAM, the cultures grew up just like the controls. results suggest that the Boric acid is efficient for ***flavus*** inhibition. The pH determined at the 3 concentrations was 6.0 and obviously does not in with fungi growth. So it turns out evident that the of this additive as inhibitor of growth and/or AT p tion by these fungi may be promising suggesting t additive may be ammended to foods and feeds sub this kind of toxigenic fungi.

Table 1
Boric Acid effect on the development and aflatoxin production *A. parasiticus* (UNBF A 12 & NRRL 2999) and *A. flavus* (NRRL 5520 & 3251)

Samples	Additive concentration (%) (a)	pH	Colony diameter (mm)	Mycelium pigmentation	Sporulation degree (b)	Spores coloration	AT Productic Intensity (c)	Cc
	0	7,0	49	Orange	3	green yellow	4	viole
A. parasiticus	0.25	6,0	5	-	0	-	0	
UNBF A 12	0,5	6,0	0	-	0	-	0	
	1,0	6,0	0	-	0	-	0	
	0	7,0	45	Orange	3	green	3	viole
A. parasiticus	0.25	6,0	5	-	0	-	0	
NRRL 2999	0,5	6,0	0	-	0	-	0	
	1,0	6,0	0	-	0	-	0	
	0	7,0	45	Orange	3	green	3	viole
A. flavus	0.25	6,0	5	-	0	-	0	
NRRL 5520	0,5	6,0	0	-	0	-	0	
	1,0	6,0	0	-	0	-	0	
	0	7,0	43	Orange	3	brawn	3	violet
A. flavus	0.25	6,0	12	Orange	0	-	2	
NRRL 3251	0,5	6,0	0	-	0	-	0	
	1,0	6,0	0	-	0	-	0	

(a) Average of the repetitions on CAM after 5 days incubation at 28° C. (b) Sporulation degree - 0 to 3.
(c) Fluorescent intensity 0 - without fluorescence 4 strong.

Table 2
***Aspergillus parasiticus* (UNBF A 12 & NRRL 2999) and *A. flavus* (NRRL 3251 & NRRL 5520) developed on acidified CAM with HCL (a)**

Samples	pH	Colony diameter (mm)	Mycelium pigmentation	Sporulation degree (b)	Spores coloration	AT Production Intensity (c)	Color
A. parasiticus	7,0	45	orange	3	green yellow	4	violet blue
UNBF A 12	6,0	45	orange	2	green yellow	4	violet blue
A. parasiticus	7,0	45	orange	3	green	4	violet blue
NRRL 2999	6,0	42	orange	3	green	4	violet blue
A. flavus	7,0	42	orange	3	green	3	violet blue
NRRL 5520	6,0	38	orange	3	green	3	violet blue
A. flavus	7,0	45	orange	3	brawn	3	violet blue
NRRL 3251	6,0	45	orange	3	brawn	3	violet blue

(a) Average of the repetitions on CAM after 5 days incubation at 28° C. (b) Sporulation degree - 2 (moderate), 3 (strong).
(c) Fluorescente intensity: 3 (intense), 4 (strong).

Table 3
Boric Acid effect on the development and the Aflatoxin (AT) production by *Aspergillus flavus* (NRRL 5520 & NRRL 3251) and *A. parasiticus* (UNBF A 12 & NRRL 2999) on YES medium (a and b)

Samples	Boric Acid (%)	Mycelium dry weight (mg/flask)	AT (ug/ flask)	Specific production AT (ug/AT mg) mycelium
A flavus	0	843,3	242,5	0,512
NRRL 5520	0,125	453,3	232,5	0,292
	0,25	323,0	120,0	0,280
A. flavus	0	730,0	242,5	0,591
NRRL 3251	0,125	663,3	175,0	0,266
	0,25	580,0	57,5	0,105
A parasiticus	0	856,0	197,5	0,288
UNBF A 12	0,125	690,0	127,5	0,184
	0,25	760,0	62,5	0,082
A parasiticus	0	906,6	120,0	1,331
NRRL 299	0,125	956,0	850,0	0,884
	0,25	580,0	42,5	0,075

(a) Average of the repetitions.
(b) Stationary culture by 14 days at 28° C.

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