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UTILIZATIION OF ORGANIC ACID FOR INHIBITING AFLATOXIN PRODUCED BY *Aspergillus flavus* IN STORED YELLOW CORN .

Abdel-Moneim, Ebtesam[1]; M.F. Emara; [2] Reham, M. El-Tookhy[2]
[1] Biochemistry Department, Faculty of Agriculture, Cairo University.
[2] Central Lab. for Food and Feed, Agricultural Research Center.

ABSTRACT

The effect of organic acid mixtures on the growth of *Aspergillus flavus* and subsequently on the production of aflatoxins B_1, B_2, G_1, G_2 and total aflatoxins in yellow corn at two moisture levels, was investigated. Yellow corn grains was calibrated to moisture levels 15 and 20% and treated with combined application of propionic acid + acetic acid + formic acid at ratios 1:1:2 (mixture1), 1:2:2 (mixture2) and 1:2:3 (mixture3). A preliminary stepwise protocol starting from 0.2% to 1.0% concentration was used to determine the concentration of organic acid mixtures that can inhibit growth of *A. flavus* in yellow corn during 21 days. Then, the chosen concentrations were used in the main experiment which lasted 60 days. In yellow corn with 15% moisture, one concentration (0.2%) was used to the main trial. When moisture was raised to 20%, two concentrations were used (0.8 and 1.0%) to the main experiment. The trial included two groups of treatments, with and without *A. flavus* infection then, stored at room temperature 30 ºC and 25 ºC for 60 days. Results showed that *A. flavus* production increased with increasing moisture level, but decreased as the concentration of organic acid increased. Total fungal count increased during the whole period of the experiment in the control groups. At all storage conditions, mixture 3 showed less fungal inhibity effect. raising moisture content of yellow corn was up to 20%, mixture 2 was found to be the best mold inhibitor at a concentration of 0.8%. Incubation at 25ºC was more effective than that at room temperature to reduce total aflatoxins. It could be concluded that, using 1.0 % of mixture 1 and mixture 2 as preservatives inhibited both growth of *A. flavus*, and aflatoxin production in yellow corn stored whether at 25°C or ats room temperature during the 21 days of storage.

Keywords: Yellow corn, Aflatoxin, *Aspergillus flavus*, Propionic, Acetic, Formic.

INTRODUCTION

Large amounts of food and feed are lost annvally due to spoilage by moulds and yeasts (Helena Lind *et al*., 2004). Corn grains are often harvested at a moisture content which may enhance the growth, colonization and mycotoxins production by a range of fungi primary *Aspergillus* and *Ppenicillum* spp. Because *Aspergillus* and *Penicillum* spp. grow actively when grain moisture is greater than 16% drying of grain to less than 15% moisture after harvest is commonly used to control fungal growth (White and Toman, 1995). Delaying drying of grains was found to cause harmful effects for such grainss (Marine *et al*., 2002). Aflatoxins are a group of toxic, mutagenic and carcinogenic compounds (IARC, 1993).They are produced by toxigenic strains of *Aspergillus flavus* and *A. paraciticus* as secondary metabolites. Various foods may support the growth of these microorganisms and consequently lead to contamination with aflatoxins (Wood, 1989 and Ellis, 1991).The contamination may take place during growth, harvest, transportation and storage and thus is difficult to be prevented (Abdelhamid,

2000). There are at least 16 naturally occurring aflatoxins, and the four major toxins are B_1, B_2, G_1 and G_2 (Rusul *et al*., 1987 and Abdelhamid, 1998). Aflatoxin B_1 is a potent hepatocarcinogen and mutagen (Heathcote, 1984 and Abdelhamid *et al.,* 2002).One alternative to prevent or reduce aflatoxin production in stored grains for animal consumption, is the use of chemical fungal inhibitors, such as the salts of propionic acid (Abdelhamid *et al.,* 1985 and Moreno et al., 2000).Volatile organic aicds such as acetic and propionic are commonly used in the food industry as preservatives (Rusul *et al.,* 1987). Propionic acid followed by acetic acid were the most potent antifungals (Helena Lind *et al*., 2004). Acetic acid is more effective in limiting yeast and bacterial growth than mold growth (Doores, 1983). Propionic acid and the propionates are highly effective mold inhibitors but have little or no effect against yeasts, which is why these chemicals are used in the baking industry (Sauer, 1977). Formic acid is the most suitable chemical preservative for feeds because of its high bactericidal and fungicidal properties (Tagaev *et al.,* 1985). It can be used alone or in combination with other acids and it has no adverse effect on farm animals. Combined application of propionic acid + acetic acid at 1 : 1 ratio (1.0, 2.5, 5.0%) completely inhibited aflatoxin B_1 production (Kavita Waghray and Reddy, 2000).The objectives of this study were to investigate the effectiveness of different mixtures of propionic, acetic and formic acids as preservatives and to determine their effect on growth and aflatoxin production by molds under different storage conditions.

MATERIALS AND METHODS

Design of the experiment:

A stepwise preliminary experiment was carried outto determine the concentration of organic acids, mixtures that can inhibit growth of *Aspergillus flavus* in treated yellow corn during 21 days. Then, the chosen concentrations were used in the main experiment which lasted 60 days. Different concentrations of the examined organic acids, mixtures were prepared using autoclaved distilled water as a diluent. Five concentrations were used for this experiment (0.2, 0.4, 0.6, 0.8 and 1.0 %) at two moisture contents of yellow corn (15% and 20%). Three organic acids, mixtures were used in the experiment according to the ratio of propionic : acetic : formic. These ratios were 1: 1: 2, 1: 2: 2 and 1: 2: 3 for mixture 1, 2 and 3, respectively. An amount of 14.4 Kg of yellow corn 14% moisture was used for the whole experiment as follow:

Moisture content of 2.4 kg yellow corn was adjusted to be 15%, and then equally divided into 24 sterile Erlenmeyer flasks; each flask contained 100 gm of yellow corn sample. These flasks were divided into 2 groups.

In group 1, yellow corn (16 flasks) was infected by *Aspergillus flavus* and divided into 2 subgroups according to incubation temperature. The first subgroup (8 samples) was incubated in incubator at 25 ^oC, while the second subgroup (8 samples) was incubated at room temperature at 30 ^oC. Each two samples were treated the same treatment with the first tested concentration (0.2% of organic acids, mixtures). According to the obtained results, this

concentration was chosen to complete the main experiment. One flask was left without inoculation as acontrol. One sample was used to determine the growth of *Aspergillus flavus,* while the other sample was used to evaluate the aflatoxins content.

Yellow corn in group 2 (8 flasks) was divided and treated with the 0.2% organic acids, mixtures without any infection. This group was divided also to 2 subgroups according to the incubation temperature (room temperature and 25 ^{o}C). Only one sample for each treatment was used to determine the growth of *Aspergillus flavus.*

When yellow corn moisture was adjusted to be 20%, amount of 12.0 kg yellow corn was used in the experiment. Every 2.4 kg of yellow corn was divided as mentioned spreviously and treated with the tested concentration. Five mixture concentrations (0.2, 0.4, 0.6, 0.8 and 1.0%) were used as a stepwise protocol for these samples, and concentrations of 0.8 and 1.0% were shosen to complete the main experiment.

The count of *A. flavus* was determined at days 0, 7, 14, 21, 28 and 60 for all samples used for the main experiment, while, aflatoxin values were determined only in the infected samples after 21 days of inoculation.

Aflatoxin production:

Aspergillus flavus NRRRL (3145) was obtained from the Natural Research Center (Dokki, Cairo). Inoculum was prepared by inoculating tubes (1.5x1.5 cm) containing Rozebengal with spores of *Aspergillus flavus* NRRL (3145). Incubated slants were incubated for 5 days at 25 ^{o}C (Shotwell *et al*., 1966).

Infection of yellow corn:

Yellow corn was obtained from Cairo Poultry Company, Giza, Egypt and deliberately infected with *Aspergillus flavus* NRRL (3145) which was grown on Rozebengal slant and characterized by crop of green conidia. The spores were scraped by adding sterile distilled water to the surface growth on the slants and an aliquot from the resulting spore suspension (1 ml) was added to conical flasks (2 liter) containing yellow corn (previously autoclaved to prevent surface contamination).

Extraction of aflatoxins:

Standard of aflatoxins: B_1, B_2, G_1 and G_2 were obtained from Sigma Chemical Company (St. Louis, MO USA). The extraction of aflatoxins was conducted according to A.O.A.C. method (1990). The samples were blended with 250 ml methanol-water (55:45, v/v) and 100 ml hexane for 1 min. at high speed. The mixture was transferred to u centrifuge bottle and centrifuged for 5 min. at 2000 rpm and an aliquot from the aqueous methanol phase (25ml) was taken with chloroform into a separatory funnel shaken for (30-60 sec.), the bottom layer (chloroform) was separated and concentrated using rotary vacuum evaporator. The residue was quantitatively transferred using small volumes of chloroform. The solvent was completely removed under nitrogen and stored at 0 ^{o}C until quantitative analysis.

Determination of aflatoxins:

Aflatoxins were determined using thin layer chromatographic technique as describedby (Shanon et al., 1983 and A.O.A.C., 1990).

Determination and adjustment of the moisture content of corn before inoculation:

Moisture contents of corn samples was estimated according to (AOAC, 1998) then adjusted to be 15% and 20% according to the following equation:
Where S = the volume of water required for100 g of samples to reach the required level of moisture content.

The total fungal count was performed as follows:

Five grams of each sample were added to a 45 ml sterile saline solution in 500 ml Erlenmeyer flasks and homogenized thoroughly on an electric shaker at a constant speed. Tenfold serial dilutions were then prepared. One ml portion of three suitable dilutions of the resulting sample suspension was used to inoculate Petri dishes each containing 15 ml Rosebengal containing 0.5 mg Chloramphenicol/ml medium to inhibit bacterial growth Plate were then incubated for 5 days at 25 ^{o}C and the fungal colonies were counted (Aziz, 1998).

Criteria of acceptance:

The criteria of evaluation of antifungal activity were estimated according to European Pharmacopoeia (2001) in terms of the log reduction in the number of viable microorganisms against the value obtained for the fungal count at zero time.

RESULTS AND DISCUSSION

At initial yellow corn moisture of 15%, minimum concentration inhibited growth of *A. flavus* was 0.2% for all treatments. When the initial moisture was raised to 20%, there was a negative effect for all used preservative mixtures when concentrations of 0.2, 0.4 and 0.6 %. The effective values were 0.8 and 1.0 % for all mixtures. Based on these results, concentrations of organic acids, mixtures chosen for further experiments with the yellow corn at moisture of 15% was 0.2%. When the initial moisture was 20%, the chosen concentrations were 0.8 and 1.0 %.

Fungal activity and count:

It is clear from the obtained data (Tables 1-3) that, stotal fungal count increased during the whole period of the experiment in the control groups which, were not treated with any preservative. This result is supported by Philip *et al*., 1983, Marine *et al*., 2002 and El-Moghazy *et al*., 2003, who stated that in the absence of preservatives fungal growth is fast and obvious.

Table (1) shows the fungal growth in yellow corn containing 15% moisture and treated with 0.2% acid mixtures. The three grains mixtures have been affected after one week hence reduction of the log number by 1 in the infected and non infected samples was recorded.

Table (1): The count of *Asperagillus flavus* colonies (cfu/g) produced from an infected and non infected yellow corn containing 15% moisture treated with 0.2% various mixtures of propionic, acetic and formic acids and stored at room temperature and 25°C.

Duration of incubation (Day)	Control		Mixture 1		Mixture 2		Mixture 3	
	Infected	Non infected	Infected	Non infected	Infected	Non infected	Infected	Non infected
At room temperature								
0	>50x10^2	>50x10^2	65x10^2	>50x10	>50x10^2	>50x10^2	>50x10	>50x10^2
7	18x10	22x10	1x10	4x10	4x10	4x10	5x10	5x10
14	16x10	23x10	1x10	1x10	2x10	-ve	3x10	3x10
21	8x10	8x10	-ve	-ve	1x10	-ve	1x10	1x10
28	12x10	6x10	-ve	-ve	-ve	-ve	-ve	1x10
60	12x10	6x10	-ve	-ve	-ve	-ve	-ve	1x10
At 25°c								
0	>50x10	50x10	>50x10^2	>50x10^2	<50x10	>50x10^2	<50x10	<50x10
7	13x10	12x10	1x10	2x10	4x10	4x10	5x10	6x10
14	18x10	17x10	-ve	1x10	10x10	2x10	3x10	6x10
21	11x10	4x10	-ve	-ve	6x10	1x10	3x10	6x10
28	37x10	18x10	-ve	-ve	2x10	-ve	-ve	2x10
60	16x10	11x10	-ve	-ve	2x10	-ve	-ve	1x10

-ve=negative

After three weeks of storage, no *A. flavus* growth was found in yellow corn treated with mixtures 1 and 2, except those non infected and treated with mixture 2. When yellow corn was incubated at 25°C, the effect of mixture 2 was declined. No complete inhibition was found in grains treated with mixture 2 during 60 days. Mixture1(25% propionic acid) was found to be the best preservative, hence log number was reduced by 1 after one week of the inoculation and complete inhibition was occurred after three weeks. This result may indicate that propionic acid has a superior antifungal effect. Gowda *et al*., 2004 stated that propionic acid at 0.1-0.5%completely inhibited *Aspergillus parasiticus* growth. Higgins and Brinkhaus, 1999 demonstrated that propionic acid showed the highest effect against moulds with the effective concentrations ranging from 0.05 to 0.25%; whereas acetic acid required concentrations of 10 lb/ton or more for effective mould inhibition.

At all storage conditions, mixture 3 has a weak inhibition effect on *A. flavus*. This may due to the lowest percentage of propionic acid (16.66%) and the highest percentage of formic acid (49.99%) in mixture 3. This is in agreement with Holmberg *et al*., 1989a who isolated *A. flavus* or *A. parasiticus* originating from acid-treated feed grain. Formic acid was less effective than propionic acid in inhibiting fungal growth. No growth was found on plates with 0.3% propionic acid, but on plates with 0.3% formic acid growth was observed in 21% of the isolates, all originating from formic acid-treated grain. Holmberg *et al*., 1989b stated that, growth of *A. parasiticus* and aflatoxin production was restricted in propionic acid-treated grain. In grain treated with formic acid alone, *A. parasiticus* totally dominated the fungal flora and produced high levels of aflatoxins.

At 20% moisture content in yellow corn, mixture 2 followed by mixture 1 were found to be the best mold inhibitors at a concentration of 0.8% (Table 2).

Mixture 2 inhibited completely the growth of *A. flavus* after 7 days in the stored yellow corn except those infected and stored at room temperature which the complete inhibition was found after 14 days. Reduction in mold count by using mixtures 1 and 2 was greater than the reduction caused by mixture 3.

Table (2): The count of *Asperagillus flavus* colonies (cfu/g) produced from an infected and non infected yellow corn containing 20% moisture treated with 0.8% various mixtures of propionic, acetic and formic acids and stored at room temperature and 25°C.

Duration of incubation (Day)	Control		Mixture 1		Mixture 2		Mixture 3	
	Infected	Non infected	Infected	Non infected	Infected	Non infected	Infected	Non infected
At room temperature								
0	$12x10^3$	68x10	10^2	32x10	10^3	21x10	$94x10^2$	49x10
7	$>10^3$	10^3	18x10	1x10	60x10	-ve	10^2	-ve
14	10^3	10^3	10x10	3x10	-ve	-ve	3x10	-ve
21	$>10^3$	$>10^3$	-ve	-ve	-ve	-ve	6x10	-ve
28	$>10^3$	$>10^3$	-ve	-ve	-ve	-ve	6x10	-ve
60	10^4	$>10^3$	-ve	-ve	-ve	-ve	-ve	-ve
At 25°c								
0	10^3	68x10	$20x10^2$	26x10	100x102	50x10	91x102	66x10
7	10^3	10^3	1x10	1x10	-ve	-ve	17x10	1x10
14	10^3	10^3	-ve	-ve	-ve	-ve	1x10	-ve
21	10^3	$>10^3$	-ve	-ve	-ve	-ve	4x10	-ve
28	10^3	$>10^3$	-ve	-ve	-ve	-ve	4x10	-ve
60	10^4	$>10^3$	-ve	-ve	-ve	-ve	-ve	-ve

-ve=negative

The effect of raising the chemical preservatives concentration to 1.0 % is presented in table (3). The *A. flavus* colonies were prevented completely after 7 days in all samples treated with mixture 1. Using mixture 2, reduced the log number by 1 after 7 days and completely removed all colonies after 14 days. Mixture 3 showed the same weak effect even after raising its concentration from 0.8 to 1.0% (tables 2 and 3).

Table (3): The count of *Asperagillus flavus* colonies (cfu/g) produced from an infected and non infected yellow corn containing 20% moisture treated with 1.0% various mixtures of propionic, acetic and formic acids and stored at room temperature and 25°C.

Duration of incubation (Day)	Control		Mixture 1		Mixture 2		Mixture 3	
	Infected	Non infected	Infected	Non infected	Infected	Non infected	Infected	Non infected
At room temperature								
0	$96x10^2$	73x10	$98x10^2$	10^2	10^2	46x10	10^2	$21x10^2$
7	10^3	10^3	-ve	-ve	73x10	1x10	3x10	5x10
14	$>10^3$	10^3	-ve	-ve	-ve	-ve	1x10	-ve
21	$>10^3$	$>10^3$	-ve	-ve	-ve	-ve	2x10	-ve
28	$>10^3$	$>10^3$	-ve	-ve	-ve	-ve	-ve	-ve
60	$>10^3$	$>10^3$	-ve	-ve	-ve	-ve	-ve	-ve
At 25°c								
0	$101x10^2$	$46x10^2$	10^2	49x10	10^2	20x10	10^2	72x10
7	10^3	10^3	-ve	-ve	6x10	-ve	66x10	1x10
14	$>10^3$	10^3	-ve	-ve	-ve	-ve	19x10	-ve
21	$>10^3$	$>10^3$	-ve	-ve	-ve	-ve	-ve	-ve
28	$>10^3$	$>10^3$	-ve	-ve	-ve	-ve	-ve	-ve
60	$>10^3$	$>10^3$	-ve	-ve	-ve	-ve	-ve	-ve

-ve=negative

Biosynthesis and accumulation of aflatoxins:

Data in tables 4 and 5 indicated that biosynthesis and accumulation of aflatoxins were mostly influenced by the concentration of mixture, incubation temperature and type of mixture. Generally, after 21 days of incubation, an increase of preservative concentration from 0.8% to 1.0% was associated with a decrease of amount of aflatoxins B_1, B_2, G_1, G_2 and total aflatoxins.

There were no differences observed in amount of aflatoxin B_1, B_2, G_1 and G_2 produced by cultures growing either at room temperature in an incubator at 25°C. In general, incubation at 25°C was the more be effective than room temperature to reduce total aflatoxin in stored yellow corn. This may due to the room temperature which was more than 25°C during summer period.

It is clear that, aflatoxins B_1, B_2, G_1, G_2 and total aflatoxins in the control were higher than those of other treatments. Aflatoxins of yellow corn treated with 0.8% organic acid mixtures are shown in Table (4).

Mixture 2 was the best preservative to inhibit the accumulation of all types of aflatoxins and total aflatoxins. No accumulation of aflatoxin B_1, B_2 and G_1 was found in yellow corn treated with mixtures 1 and 2 under 25°C incubation. The lowest total aflatoxin values were found in the yellow corn treated with mixture 2 (3.94 and 24.71 ppb at 25 °C and room temperature, respectively). Raising the mixture concentration to 1.0 % (Table 5), showed no accumulation of aflatoxin B_1 and B_2 was in yellow corn treated with any of the three mixtures either incubated at room temperature or 25 °C. This is in agreement with Verma *et al*., 2000 who indicated that aflatoxin B_1 biosynthesis increased with increasing moisture levels, and decreased as the concentration of organic acids increased.

Table (4): Accumulation of aflatoxin (AN) B1, B2, G1, G2, and total aflatoxins (ppb) in an infected yellow corn containing 20% moisture treated with 0.8% various mixtures of propionic, acetic and formic acids under different incubation conditions after 21 days of incubation .

Type of AN	Treat	Control	Mix 1	Mix 2	Mix 3
B1	Room Temperature	491.01	31.49	-	-
	25 ºC	662.75	-	-	31.58
B2	Room Temperature	15.41	16.7	-	-
	25 ºC	111.17	-	-	9.97
G1	Room Temperature	833.90	32.69	17.44	25.11
	25 ºC	1559.24	-	-	57.90
G2	Room Temperature	258.06	17.57	7..27	7.95
	25 ºC	325	9.01	3..94	14.42
Total aflatoxins	Room Temperature	1738.38	98.45	24.71	33.06
	25 ºC	2658.16	9.01	3..94	13.87

Table (5): Accumulation of aflatoxin (AN) B1, B2, G1, G2, and total aflatoxin (ppb) in an infected yellow corn containing 20% moisture treated with 1.0 % various mixtures of propionic, acetic and formic acids under different incubation conditions after 21 days of inoculation.

Type of AN	Treat	Control	Mix 1	Mix 2	Mix 3
B1	Room Temperature	780.28	-	-	-
	25 ºC	694.19	-	-	-
B2	Room Temperature	294.34	-	-	-
	25 ºC	142.19	-	-	-
G1	Room Temperature	1542.18	25.81	57.90	47.44
	25 ºC	2218.50	-	-	31.39
G2	Room Temperature	681.81	10.54	6.53	8.62
	25 ºC	530.90	11.07	11.65	6.74
Total aflatoxins	Room Temperature	3298.6	36.65	64.43	56.06
	25 ºC	3585.78	11.07	11.65	38.13

Generally, the results showed that mixtures 1 and 2 were the most effective preservatives against total aflatoxins and aflatoxin B_1. This result may due to the high percentages of propionic acid in mixture 1 (1 propionic : 1 acetic : 2 formic) and mixture 2 (1 propionic : 2 acetic : 2 formic).

Propionic acid at 0.05-0.5% completely inhibited aflatoxin production (Gowda *et al*., 2004). Concentrations over 60 ppm undissociated propionic acid concentration did not show an increase in aflatoxin G_1 and aflatoxin B_1 indicating that there is no need to increase the concentration of propionic acid over this value (Molina and Giannuzzi, 2002). At 10% moisture levels, 0.5% propionic acid and 1% acetic acid were effective in preventing the growth of *A. flavus* and subsequent aflatoxin B_1 production. Propionic acid was the

most effective in the prevention of aflatoxin B_1, followed by acetic acids (Verma *et al*., 2000). Kavita Waghray and Reddy, 2000 stated that individual or combined application of propionic acid and acetic acid at 1.0, 2.5 and 5.0% completely inhibited aflatoxin B_1 production by *Aspergillus flavus* on maize.

It could be concluded that, during 21 days of storage, using 1.0 % of mixture 1 (1 propionic : 2 acetic : 2 formic) and mixture 2 (1 propionic : 2 acetic : 2 formic) as preservatives inhibited both growth of *A. flavus*, and its aflatoxin production on yellow corn stored at 25°C or at room temperature.

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استخدام بعض مخاليط الأحماض العضوية لتثبيط انتاج الافلاتوكسينات الناتجة من فطريات *Aspergillus flavus* فى الأذرة الصفراء تحت ظروف التخزين المختلفة.

ابتسام عبد المنعم[1] ، محمد عمارة[2] و ريهام محمود الطوخى[2]

1 قسم الكيمياء الحيوية - كلية الزراعة - جامعة القاهرة.

2 المعمل المركزى للأغذية و الأعلاف - مركز البحوث الزراعية.

فى هذه التجربة تم دراسة تأثير المخاليط الأحماض العضوية على نمو فطريات *Aspergillus flavus* وإنتاج الأفلاتوكسينات B_1,B_2,G_1,G_2 والأفلاتوكسينات الكلية وذلك في حبوب الأذرة عند مستويين مختلفين من الرطوبة. تم ضبط الرطوبة في الأذرة الصفراء عند 15% و20% ثم تمت معالجتها بمخاليط من أحماض البروبيونيك والخليك والفورميك بنسب 1:1:2 (مخلوط 1) و 1:2:2 (مخلوط 2) و 1:2:3 (مخلوط 3). ولقد تم إجراء تجربة تمهيدية بصورة تصاعدية تدريجية لتحديد تركيزات مخاليط الأحماض العضوية التى تثبط نمو فطر *Asperillus flavus* في الأذرة الصفراء خلال 21 يوماً ثم تم استخدام هذه التركيزات في التجربة الرئيسية ةالتى استمرت 60 يوماً في الأذرة الصفراء التى تحتوي على 15% رطوبة تم استخدام تركيز واحد (0.2%) في التجربة الرئيسية وعند رفع الرطوبة إلى 20% تم استخدام تركيزين (0.8% ، 1%) في التجربة الرئيسية. والتجربة اشتملت على مجموعتين من المعاملات إحداها تم حقنها بفطر *A. flavus* والأخرى بدون حقن ثم بعد ذلك تم تخزينها إما على درجة حرارة الغرفة (30°م) أو على درجة حرارة 25°م لمدة 60 يوماً.

أثبتت النتائج أن *A. flavus* زاد بزيادة نسبة الرطوبة لكنه قل بزيادة تركيز الأحماض العضوية. وقد زاد العدد الكلي للفطريات في المعاملة الكنترول عن جميع المعاملات خلال فترة الأحماض ، وتحت جميع ظروف التخزين كان المخلوط 3 هو الأضعف في تثبيط نشاط *A. flavus*. عند رفع محتوى الأذرة من الرطوبة إلى 20% كان المخلوط 2 هو الأفضل كمثبط للفطريات عند تركيز 0.8%. وعند تخزين الأذرة الصفراء على 25°م كان تأثير الأحماض العضوية على تقليل الأفلاتوكسينات الكلية أكثر فاعلية منه عند التخزين على درجة حرارة الغرفة. ويمكن التوصية بأن استخدام مخلوط 1 ومخلوط 2 بتركيز 1% كمادة حافظة لا يثبط فقط نمو فطر *A. flavus* ولكن أيضاً يثبط إنتاج الأفلاتوكسين في الأذرة الصفراء التى تخز على درجة حرارة 25°م أو درجة حرارة الغرفة خلال 21 يوماً من التخزين.