Effect of container , medium weight , and moisture content on aflatoxin B1 production on rice

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تاريخ الاستلام: 2011/2/11

Abstract

Laboratory experiment was carried out to evaluate three kinds of media containers for aflatoxin B1 production on rice . One liter volume flasks , one kilogram silicon bag, and 25 Cm wide ,5Cm high Petri dish ,with three media weight , 50 , 150 and 250 gram / container at three moisture content , 18% , 20% , 22%. Results showed highly significant differences ($P< 0.01$) in amount of aflatoxin B1 concentrations between treatments , and the Petri dish was superior among treatments followed by flask while silicon bag resulted in the least of aflatoxin production at 18% of moister content for all used weights . The results also showed adverse effects of increased weight and moisture content of culture media on fungal growth and inhibition of toxin production in all treatments .

تاثير الحاوية ووزن الوسط والمحتوى الرطوبي في انتاج سم الافلا B1 في الرز

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الخلاصة

اجريت تجربة مختبرية لتقييم ثلاثة انواع من حاويات الوسط الزرعي على انتاج سم الافلا B1 في الرز , دوارق زجاجية حجم 1 لتر, واكياس السليكون الحراري حجم 1 كيلوغرام واطباق بتري زجاجية قطر 25 و ارتفاع 5 سنتمتر عند مستويات رطوبية 18% , 20% و 22% وبثلاث اوزان 50 , 150 , 250 غرام / حاوية . اظهرت النتائج وجود فروقات معنوية عالية ($P<0.01$) في كمية سم الافلا B1 المنتجة بين المعاملات وتفوقت معاملة الاطباق على بقية المعاملات تلتها معاملة الدوارق الزجاجية ثم اكياس السليكون الحرارية عند المستوى الرطوبي 18% ولمختلف الاوزان المستعملة . اظهرت النتائج ايضا التاثير السلبي لزيادة الوزن و المحتوى الرطوبي للوسط الزرعي في نمو الفطر و انخفاض كمية السم المنتج وفي جميع المعاملات.

Introduction

Mycotoxins are secondary metabolites of fungi , have adverse effects on human and animals (4) .Aflatoxins are the most concerned kind of mycotoxins because of word wide distribution and the growth of producing fungi at wide range of environmental conditions such as moisture and temperature (2, 18). Since the initial discoveries of mycotoxins which indicated the death of hundreds thousands of turkey poults in 1960 , Aflatoxin B_1 which is produced by many strains of *Aspergillus flavus* and *A.parasiticus* is known to be the most significant form of mycotoxins that causes serious risk to animals and human health (3, 12 ,13) . The production of toxin by fungi isolates is basically genetic dependent , and then influenced by culture conditions. Aflatoxin has been artificially produced on peanut , wheat bran , corn meal, soybeans , sorghum and rice, and it was found that rice was the best substrate for aflatoxin production . Rice substrate is cheap , and the fermentation has no interference in the common analytical methods . The toxic kernels are easily grounded to a fine particles and the yield of toxin is good enough so that the amount of toxic rice powder added to the diet is usually too small to alter the nutrient quality(11,17). Since The quantities of aflatoxin needed for consumption during feeding experiments are so large therefore any improvement in yield would be appreciated in savings effort and expense (1). However large quantities of aflatoxinB_1 must be produced biologically for chemicals studies and large scale feeding trials . Little information is known about the effect of container in growth of fungi and aflatoxin production, therefore the objective of the present experiment was to evaluate the influences of media containers, weight of medium and humidity leveles on aflatoxin production on rice media.

Materials and methods

Three factorial experiments were carried out in plant pathology laboratory, College of Agriculture , AL-Anbar Univ, to evaluate the influence of three kinds of containers ,weight and moisture content of media in aflatoxin production.

Culture and inoculum . *Aspergillus flavus* strain (AF10) was obtained from mycotoxin laboratory – college of Agriculture – Baghdad Univ. The inoculum was prepared by inoculation the isolate on sterilized corn seeds at $25C^0$ for 1 week to have mass of spores that used as initial inoculum for treatments . Three kinds of containers were used in this investigation as the following .

(1) 1 liter volume flasks .

(2)1 kilo gram size (kg) silicon bag .

(3) Petri dishes 25 centimeters (cm) diameter and 5 cm high .

Three weights of rice were used for each kind of container, 50 , 150 and 250 grams (gm) with three levels of moisture contents for rice substrate 18 , 20 , 22% by using rice seeds with 11% moisture content , which was determined by oven method and an adequate quantity of water was added in order to obtained moisture levels 18 , 20 , and 22% . A high relative humidity (95%) was provided for all treatments(7 , 23) .All treatments were replicated three times . The substrate (rice seeds) was placed in the containers , sealed and autoclaved for 15 min at 20 psi .

Autoclaving was repeated after 48 hours to ensure better stirilization (8, 9). After cooling the media of all treatments were inoculated with spores of fungal isolate as contaminated corn seeds (15 seeds were added for each treatment). The containers were shaked vigorously to assure homogenized distribution of spores in the media, and incubation was maintained at $15C^0$ for the first 24 hours then the temperature was raised to 28 C^0 for 1 week (22) . After incubation , the samples were transferred to an oven for drying at $50C^0$.The aflatoxin was then extracted , an amount of 50gm samples representing each treatment were placed into 500ml glass-stoppered Erlenmeyer flask and mixed with 25 ml water and 250 ml chloroform , shaked for 30 minutes on wrist- action shaker. The samples were filtered through a fluted paper and the first 50 ml of filtrate was collected (15).

Quantification of aflatoxin was made by thin layer chromatography (TLC) , purified filtered extracts and standards were spotted on TLC . The spots of B_1 were compared as fluorescent intensities with those of standard B_1 spots to determine which sample spot that matches the standard spots . The semi quantities of aflatoxin was achieved depending on the formula illustrated below (10) :

$$W = \frac{V_1 V_2 Ps V_5}{m V_3 V_4} \text{ (mg / kg)}$$

Where W = sime quantities of aflatoxin B1
where m= mass of sample extracted
ps = mass concentration of standard B1 solution (mg/ L)
V_1 = volume of spot of B1 standard with same intensity
as sample spot.
V_2= volume of final dilution of sample extract
V_3= volume of sample spot whose B1 intensity matches
that of B1 standard Spot of volume V1
V_4= volume of chloroforme extract chromate graphed in sample.
V_5= total volume of chloroform employed in sample .
Examination was made under high intensity ultraviolet light .
Experiments were replicated three times and results were reported
as averages.

Results and discussion

The results reported for the 50 gms amount of media for each container showed a significant differences in mean concentration of aflatoxin production (Table.1) . Aflatoxin concentrations were 1.8µg / g and 1.9 µg / g for the flasks and petri dishes container respectively , while the concentrations was 1.5µg/ g for the silicon bag container , at 18% moisture content of culture media . Differences between concentrations means for the three treatments were significantly increased with increasing level of moisture content , which were 1.8 , 1.4 µg / g and 1.9, 1.6µg / g for flask and petri dish treatment at 18 , 22% respectively . No significant differences were observed between means of concentrations at 18 , 20% for both treatments . Results also indicated that no significant differences in means of aflatoxin concentrations in silicon bag samples at moisture content of 20 , 22% ,which was 1.2 µg / g for both , while it was1.5 µg / g at 18% of moisture content .

When a 150 gm of media was used for each container , results showed highly significant differences (P < o.o1) in means of aflatoxin concentrations between and within treatments according to the variations of moisture content in culture media (table . 2) . The means of concentrations were 1.7 , 1.6 , 1.3 µg / g in flasks treatment ; 1.5, 1.3, 1 µg / g in silicon bag and 1.9 , 1.6 , 1.4 µg / g in Petri dishes at 18% ,20% and 22% moisture contents respectively . These results are indicating negative effects of moisture content above 18% on aflatoxin concentrates . This effect could be attributed to low level of oxygen available for fungus growth with increasing level of moisture which subsequently influence toxin production.

The bioactive process for fungus would contribute in the increasing the level of moisture . Results presented in this study showed agreement with those of (Christensen (6) and Vincent (21))whom found that the moisture content at 18% was favorable for fungus growth and aflatoxin production. The results showed that the highest mean of aflatoxin concentrate was obtained in petri dishes treatment. Level of detectable aflatoxin was 1.9µg / g in petri dishes follow by flasks treatment with 1.7µg / g while the amount was 1.5 µµg /g in silicon bag treatment . The same trend of aflatoxin production was observed when the media weight was increased to 250 g for each container (table . 3) . The treatment of petri dishes was also superior in average of aflatoxin production , which was 1.8 µg / g while the concentrations were 1.4 and 0.6 µg / g for flask and silicon bag treatments . This reduction in mean of aflatoxin production in the last two treatments might be attributed to the relatively high contamination with yeast in culture media due to the difficulties of silicon bag sealing and handling with 250 g weigh of media . Another factor could be referred to the shortage of oxygen , since the culture media become too dense to allow an adequate breathing of fungal strain(5, 14 , 19 ,22) . Besides, the growth of fungal strain was limited in the upper side of media and never been interpenetrated in depth . The results also indicated that means of aflatoxin concentrate was significantly reduced (P < 0.01) in petri dishes treatment at 22% of moisture content, and this would strengthen the thought of the negative effect of moisture content when increased over 18% , (16 , 20 , 23).

Results of the present experiment showed that the petri dishes treatment was the most preferred container for small scale aflatoxin production because the containers were more easy for handling in case of introducing and removing of the media in comparison to the flasks and silicon bag . Therefore this container is recommended for obtaining large quantity of aflatoxin to be used in scientific experiments with less effort and time . Petri dishes container method is effectively used with250g weight of culture media at 18% of moisture content .

Table 1. Mean concentration of aflatoxin produced in 50g of media in different containers at three levels of moisture content .

Treatment	Concentration of aflatoxin µg / g		
	Moisture content		
	18%	20%	22%
Flask	1.8	1.7	1.4
Silicon bag	1.5	1.2	1.2
Petri dishes	1.9	1.8	1.6
LSD (P< 0.01) for treatments (0.265) for moisture contants (0.265) for interaction(o.45)			

Table 2. Mean concentration of aflatoxin produced in 150g of media in different containers at three levels of moisture content.

Treatment	Concentration of aflatoxin µg / g		
	Moisture content		
	18%	20%	22%
Flask	1.7	1.6	1.3
Silicon bag	1.5	1.3	1
Petri dishes	1.9	1.6	1.4
LSD (P< 0.01) for treatments (0.298) for moisture contants (0.298) for interaction(o.43)			

Table 3. Mean concentration of aflatoxin produced in 250g of media in different containers at three levels of moisture content.

Treatment	Concentration of aflatoxin µg / g		
	Moisture content		
	18%	20%	22%
Flask	1.4	o.9	0.7
Silicon bag	0.6	o.4	0.1
Petri dishes	1.8	1.6	1.2
LSD (P< 0.01) for treatments (0.450) for moisture contants (0.450) for interaction(o.78)			

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