INFLUENCE OF SOLAR SIMULATOR, GAMMA IRRADIATION AND LASER RAYS ON THE GROWTH AND AFLATOXIN PRODUCTION OF ASPERGILLUS FLAVUS AND ASPERGILLUS PARASITICUS

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ABSTRACT

One hundred samples of processed animal feeds, 50 milk samples from cases of mastitis and 30 vaginal swabs from cases of abortion in sheep and cattle were collected from farms at Giza Governorates. The samples were collected from diseased animals showing different clinical manifestation including diarrhea and pneumonia in calves, cattle and sheep; mastitis and some cases of abortion. The mycological examination of these samples revealed the isolation of fungi belonging to 7 genera of moulds and 2 genera of yeasts. The rates of isolation of Aspergillus flavus from animal feeds, mastitic milk and vaginal swabs were (80%, 50% and 50%), respectively, while the rates of isolation for Aspergillus parasiticus were (35%, 24% and 10%), respectively. Aflatoxins were detected in 60% and 40% of feed and mastitic milk, with the mean levels of $(110\pm3.5 \text{ and } 10\pm0.2 \text{ ppb})$, respectively. The isolated strains of A. flavus and A. parasiticus were screened for AFB₁ production before and after exposure to doses of gamma radiation and photodynamic inactivation for evaluation of their effect on fungal growth and toxin production. The doses 4 kg of gamma radiation were effective to prevent spore germination and mycelium growth of both A. parasiticus and A. flavus. Whereas, AFB₁ production was inhibited at a dose of 2 and 3kGy, respectively. Whenever, the rays of solar simulator and light emitting diodes (LED) in the presence of phloxine B as photosensitizer caused complete inhibition of mycelium growth and AFB₁ production at a dose level of 2.0 mg% phloxine B in case of solar simulator. On the other hand, the application of LED resulted in complete inhibition of mycelium growth and AFB₁ production at a dose level of 1 and 2 mg% phloxine B. The economical and health significance of the present results were fully discussed.

Key words: Solar simulator; gamma irradiation; laser rays; AFB1; A. flavus

INTRODUCTION

Mycotoxicosis due to environmental pollution of feeds and water by toxigenic fungi constitute a serious animal health and public health hazards. The most important mycotoxigenic fungi are those producing aflatoxins, which received greater attention than any of the other mycotoxins because of their demonstrated carcinogenic effects in susceptible animals and their acute toxigenic effects in human and also they are unique in being resistant to degradation under normal food processing conditions (Ciegler and Vesonder, 1983). The correlation between the environmental factors, mycosis and mycotoxicosis in animals and its role in initiation of food born infections had been reported by Hassan, (2003); Hassan and Mogeda 2003, 2004, 2007, 2008 and 2009; Abo-Al-Yazeed et al. (2008) .Several different physical and chemical approaches have been tried to detoxify mycotoxins from I feeds.

The adverse side effects of chemical compound direct the searches to find other safe natural products to control mycotoxicosis (Hassan, 2003; Hassan et al., 2007; Sayed El Ahl et al., 2006). This makes the selection of proper decontamination methods that will effectively decompose aflatoxins, while retaining the nutritive quality and palatability of the treated food a continuous challenge. One of possible approach is to use photodynamic therapy (PDT), which is a novel and promising biophotonic technology. Photodynamic therapy is an entirely new modality and its development can likened to that of the discovery of antibiotics (McCaughan, 1999). It is important to make an effort to develop safe and practical detoxification methods using different radiation types, as gamma rays, laser and solar simulator. So, the aim of the present work was to study the effect of gamma irradiation and photodynamic inactivation (PDI) on fungal growth, aflatoxin B_1 production of Aspergillus flavus and Aspergillus parasiticus.

MATERIAL AND METHODS

Materials

Samples One hundred samples of processed animal feeds, 50 milk samples from cases of mastitis in sheep and cattle and 30 vaginal swabs from cases of abortion were collected from farms at Giza Governorates in

which diseased animals suffering from different clinical manifestations including diarrhea and pneumonia in calves, cattle and sheep; mastitis and some cases of abortion. The collected samples were transported to laboratory of Animal Health Research Institute in clean sterile plastic bags .The samples were examined mycologically and the recovered isolates of *A.flavus* and *A. parasiticus* were used in this study.

Standard aflatoxins: Standards of AFB₁, B₂, G_1 and G_2 were purchased from Sigma (USA).

Photo sensitizer: Phloxine B (D & C No. 28) photo sensitizer with absorption spectrum 537 nm was purchased from Sigma (USA).

Source of Gamma radiation: Cobalt 60 gamma cell (Gamma chamber 4000A) located at the National Center for Radiation Research and Technology (NCRRT), Naser city, Cairo, Egypt

ORIEL Solar simulator: Located at National Institute of Laser Enhanced Science, Cairo University, Egypt (NILES) was used for artificial light exposure.

Green light emitting diodes (LED): Located at NILES, Cairo University, Egypt, the wave length used in this study was 530 nm and exposure time was 15 min.

Methods

Isolation and identification of moulds

Each sample of feed, milk and vaginal swabs was subjected for isolation and identification of fungi according to **Conner et al.** (1992).

Production and estimation of aflatoxins (Gabal et al., 1994)

The isolated fungi (*A.flavus and A. parasiticus*) were inoculated into flasks containing 50 ml of sterile yeast extract solution (2%) containing 20% sucrose (YES). Inoculated flasks were incubated at 25°C for 10-15 days. At the end of the incubation period, extraction and detection of produced aflatoxins was estimated by fluorometric method as recommended by (**Hansen**, 1993).

Detection of aflatoxin B1 in animal and poultry feeds

Twenty five grams of the ground feed samples were subjected for extraction and purification of toxins using immunoaffinity column and

quantitatively estimated by fluorometric method according to AOAC, (1990) and Hansen (1993).

Evaluation of the effect of different types of radiation on growth and aflatoxin B_1 production of A. flavus and A. parasiticus:

Preparation of spore suspension of A. flavus and A. parasiticus:

Aflatoxin B_1 producer strains of A. flavus and A. parasiticus were grown on potato dextrose agar slants for 10 days at 28°C. Spores were harvested in sterile 0.1% Tween 80 solution, filtered through four layers of sterile gauze. Collected spores centrifuged at 3000 x g for 5 minutes, washed three times with sterilized distilled water and then re-suspended in sterilized tween 80 solutions. The number of spores was estimated by haemocytometer and the suspension was adjusted to contain approximately 10^6 spores/ ml.

Effects of gamma radiation and PDI using solar simulator or LED on aflatoxin B1 production and mycelium dry weight

The prepared spore suspensions (10⁶spores/ml) were distributed into several sterile test tubes. Each containing 5 ml and then irradiated in a CO60 irradiator at a dose level of 0, 1, 2, 3 and 4 KGy. Whereas, in case of PDI using solar simulator or LED, 100 μl of the prepared spore suspension were mixed in tissue plates with 100 μl of different concentration of phloxine B photosensitizer (0 mg%, 0.5 mg%, 1 mg% and 2.0 mg%) (Shahin and Aziz, 1997; Aziz and Youssef, 2002; Abou Srea, 2005): Then incubated for 3 hours in dark. After that, the content of the plates were irradiated while mixing with magnetic stirrer (to obtain homogenous distribution of light) using solar simulator at fluency rate of 400 W/m² or at 530 nm wave length for 15 min in case of LED. Three wells of each concentration of photosensitized spore suspension remained without irradiations and were considered as negative control (Abou Srea, 2005).

The non-irradiated and irradiated spores suspension (200µl) were inoculated into 100 ml Erlenmeyer flasks having 25 ml of sterile yeast extract sucrose (2% yeast extract and 15% sucrose) and supplemented with 0.019% P-cresol. Inoculated flasks were incubated in the dark for 20 days. At the end of incubation period, YES medium was filtered through

a Buchner funnel fitted with pre-weighted Whattman number 1 filter paper. The mycelium was washed with 10 ml distilled water and then the filter paper with the mycelium was dried at 100°C for 48 hrs, dried in desiccators and weighed. The aflatoxin B₁ concentration of the culture filtrate was determined as described before.

Statistical analysis

The obtained date were computerized and analyzed for significance.. Calculation of standard error and variance according to SPSS 14 (2006).

RESULTS AND DISCUSSION

In the last decades the mycologists and epidemiologists throw the light on mycotoxins elaborated by wide variety of fungal species during their growth and it's contamination of feeds. This directs the attention of researches towards studying various methods to detect, prevent and control mould growth and mycotoxin production in feeds (Dalcero et al., 1997; Hassan, 1998, 2003; Hassan et al., 2004, 2007, 2009; Abo- Al-Yazeed et al., 2008). The collected processed animal feed samples from farms suffering from cases of diarrhea and pneumonia in calves and mastitis in cattle and sheep were screened for fungal contamination and detection of aflatoxin B₁.

The results revealed the isolation of fungi belonging to 7 genera of moulds and 2 genera of yeasts from feeds. The most predominant rate of isolation of moulds were belonged to genus Aspergillus (85%), particularly, A. flavus (80 %) and A. parasiticus (35%). Followed by Penicillium (76%), Mucor (60%), Rhizopus (24%) and Cladosporium (20%). The yeast cultures were identified as Candida albicans (6 %) and Rhodotorula species (20%) (Table1).

On the other hand, the isolated fungi from vaginal swabs of aborted animals and milk of mastitic animals yielded nearly the same orders of frequency, where, members of *Aspergillus species* were also at the top incidence of other isolated moulds (60% in both). Moreover, *C.albicanse and Rhodotorula sp.* were recovered at a rate of (66.6%) and (40%) from

samples of vaginal swabs of aborted animals and (73.8%) and (50%) from milk samples of mastitic animals, respectively. These differences in the level of contamination may be due to the exposure of the examined samples to different climatic condition either during preparation or transportation or storage. The yeast growth required more moisture content in the surrounding environment, so their incidence in samples of vaginal swabs and milk were relatively higher than in case of feed samples.

Table (1): Prevalence rate of fungi in feeds, milk and vaginal swabs collected from diseased animals suffering from different clinical manifestations.

Fungal species	Incidence of fungi in samples o:f						
	Vaginal	swabs of	Milk of	mastitic	Processed	animal	
	aborted animals (30)		animals		feeds		
			(5	(50)		(100)	
	No. of	%	No. of	%	No. of	%	
	(+ve)		(+ve)		(+ve)		
Aspergillus(A)	18	60	30	60	85	85	
species:							
1- A.flavus	15	50	25	50	80	80	
2-A. parasiticus	3	10	12	24	35	35	
3- A. niger	20	66.6	8	16	15	15	
4- A.fumigatus	10	33.3	9	18	20	20	
5- A.ochraceus	8	26.6	10	20	8	8	
Penicillim spp.	9	30	9	18	76	76	
Fusarium spp.	4	13.3	5	10	8	8	
Mucor spp.	6	20	15	30	60	60	
Rhizopus spp.	3	10	10	20	24	24	
Cladosporium	1	3.3	6	12	20	20	
spp.							
Alternaria spp.	1	3.3	4	8	0	0	
Scopulariopsis	2	6.6	2	4	20	20	
spp.							
Candida albicans	20	66.6	22	73.8	6	6	
Rhodotorula spp.	12	40	15	50	20	20	

^{*} % : Were calculated according to the No. of examined examined samples.

Aflatoxin B_1 received greater attention than other mycotoxins because of its demonstrable carcinogenic effect in susceptible animals and its acute toxic effect in human (**Wogan**, **1973**; **Bressac et al.**, **1991**). Therefore, the samples of processed feeds (50) and milk (25) of mastitic animals were evaluated for aflatoxin B_1 contamination. The results showed that AFB₁ was detected in 30 samples of feeds (60%) with the maximum level of (1800 ppb) and minimum level of (15 ppb) with a mean level of (110 \pm 3.5). Whereas, in the samples of milk of mastitic animals AFB₁ was detected in 10 samples (40%) with the maximum level of (15 ppb) and minimum level of (3 ppb) with a mean level of (10 \pm 0.2)(Table, 2). Whenever, the maximal level allowed by Food and Drug Administration (FDA) is (20 ppb) for all feeds and foods and (0.5 ppb) for fluid milk (**Schuller et al.**, **1983**). The detected levels of AFB1 in the present work were significantly hazard for human and animal health.

Table (2): Detection of Aflatoxin B1 in samples of feed and mastitic milk samples.

Examined	Prevalence of aflatoxins in examined samples(PPB)				
samples	Incidence		Levels of aflatoxin B ₁ in +ve samples		
	No. of	%	Max.	Min.	Mean±
	+ve				SE
Processed	30	60	1800	15	110±3.5
animal					
feeds (50)					
Mastitic	10	40	15	3	10±0.2
milk (25)					

On the other hand, the isolates of A.flavus~(100)~and~A.~parasiticus~(50) that recovered from present samples were screened for AFB1 production on synthetic medium of YES . The obtained results yielded that(70%) of A.flavus~and(40%) of $A.~parasiticus~produced~significant~levels~of~toxin~with~a~maximum~of~(235~and~210~ppb), minimum~levels~of~(30~and~10~ppb)~with~the~mean~levels~of~(190<math>\pm~4.2~$ and~115 ±0.5), respectively. These mycotxoins residues in food and feed causes carcinogenic, teratogenic, haemorrhagic and immunosuppression~effect to~human~and

animal health (Hassan, 1998, 2003; Hassan et al., 2004, 2007, 2008, 2009; Sayed El Ahl et al., 2006).

Table (3): Aflatoxin B_1 production by isolated strains of A. flavus and A.

parasiticus. On synthetic medium.

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Tested	Amount of AFB _{1 ug} / 1 of YES broth				
isolates	Incidence		Levels of aflatoxins (ug/l of YES broth)		
	No. of +ve	%	Max.	Min.	Mean± SE
A.flavus (100)	70	70	235	30	190± 4.2
A.parasit icus (50)	10	40	210	10	115±0.5

It is suggested that in all countries especially developing ones, the animal diseases increased due to the increased consumption of contaminated feed with fungal organisms and their toxins (Hassan et al., 2004, 2007, 2008, 2009; Sayed El Ahl et al., 2006). Therefore, the diverse action and diseases resulted from fungal and mycotoxin contamination enforced the continuous trials of scientists to find out a new and safe method for their control. The updated important methods include the application of rays such as gamma radiation to inhibit the growth of toxigenic strains of A. flavus and A. parasiticus and AFB₁ production in synthetic medium. The obtained results in table (4) revealed that the mycelium dry weight (g/l) for both A. flavus and A. parasiticus decreased by increasing the dose of gamma radiation and complete inhibition of fungal growth occurred at a dose of 4.0 kGy. Whereas, AFB₁ production decreased by increasing the dose of gamma radiation. The toxin could not be detected at 2 kGy in case of A. parasiticus and at 3 kGy in case of A. flavus.

The same findings were observed by Hassan (1994); Refai et al. (1996); Hassan and Aziz (1998); Aziz and Mahrous (2004) who studied the effect of gamma irradiation on the viability and production of aflatoxin by Aspergillus flavus in feed, field-dried hay and green stuff.

There was a good relationship between mycelium dry weight and the concentration of AFB₁ which significantly decreased from $(235.0\pm3.5 \,\mu\text{g/l})$ at $0.0 \,\text{KGy}$ to $(110.3\pm4.4 \,\mu\text{g/l})$ at $1 \,\text{KGy}$ and to $(15\pm0.8 \,\mu\text{g/l})$ at $2 \,\text{KGy}$, whereas the toxin could not be detected at $3 \,\text{KGy}$ in case of A. flavus. While in case of A. parasiticus the concentration of AFB₁ significantly decreased from $(210.0 \pm 1.8 \,\mu\text{g/l})$ at $0.0 \,\text{KGy}$ to $(44.8 \pm 0.5 \,\mu\text{g/l})$ at $1 \,\text{KGy}$, whereas the toxin could not be detected at $2 \,\text{KGy}$ by Chang and Markis (1982) who reported that increasing the radiation dose in the range of $0.0 \,\text{to} \,4.0 \,\text{KGy}$ resulted in decreasing aflatoxin formation in barely. Also, El- Hadi (1986); Hassanien (1987); Hassan and Aziz (1998) showed that exposure of A. flavus to low doses of gamma rays $2.0 \,\text{KGy}$ resulted in decreased fungal growth and aflatoxin production and complete inhibition occurred at a dose level of $3 \,\text{KGy}$.

Table (4): Effect of gamma radiation on growth of toxigenic strains of A. *flavus* and A. *parasiticus* and AFB₁ production in synthetic medium.

	Aspergillus flavus		Aspergillus parasiticus	
Radiation doses kGy	Mycelium dry weight (g/l of YES broth)	Aflatoxin B1 concentration (ug/l of YES broth)	Mycelium dry weight (g/l of YES broth l)	Aflatoxin B1 centration (ug/l of YES broth)
Before radiation	200.3 ± 0.69	235.0 ±3.5	220.2 ± 1.5	210.0 ± 1.8
1	106.00 ± 0.99	110.3 ± 4.4	120.00 ± 0.95	44.8 ± 0.5
2	80.7 ± 0.20	15.00 ± 0.8	54.2 ± 0.60	0.0
3	30.83 ± 0.48	0.0	30.0 ± 0.5	0.0.
4	0.0.	0.0	0.0	0.0

Table (5) Photodynamic effect of phoxine B on growth toxigenic strains of Aspergillus flavus and Aspergillus parasiticus and AFB1

production after exposure to solar simulator.

pro	A. flavus		A. Parasiticus		
		Aflatoxin B1		Aflatoxin B1	
Conc.	Mycelium dry	concentration	Mycelium dry	concentration	
of dye	weight (g/l of	(ug/l of YES	weight	(ug/l of YES	
(%)	YES broth)	broth)	(g/l of YES broth	broth)	
Before					
add of	200.3 ± 0.69	235.0 ± 3.5	220.2 ± 1.5	210.0 ± 1.8	
dye.					
0.5	1120±	80.83±	130.3±	98.6±	
0.5	3.00	1.96	3.7	4.7	
1	60.3±	27.33±	96.0±	20.2±	
1	1.93	1.88	2.92	2.1	
2.0	0.00±	0.00±	0.00±	0.00±	
	0.00	0.00	0.00	0.00	

Table (6): Photodynamic effect of Phloxine B on growth of toxigenic strains of A. flavus and A. parasitcus and aflatoxin B₁ production after exposure to LED.

	Aspsegillus flavus		Aspsegillus parasiticus	
		Aflatoxin B ₁		Aflatoxin B ₁
Conc. of	mycelium dry	concentration	mycelium dry	concentration
dye	weight (g/l of	(μg/l of YES	weight (g/l of	(μg/l of YES
(mg%)	YES broth)	broth)	YES broth)	broth)
Before				
add of	200.3 ± 0.69	235.0 ± 3.5	220.2 ± 1.5	210.0 ± 1.8
dye.				
0.10	148.00 ± 2.00	132.7 ±3.5	167.57 ± 2.62	125.3 ± 2.35
0.50	55.00 ± 1.6	65.2 ± 2.3	130.40 ± 3.10	70.10 ± 1.50
1.00	0.0.	0.0	50.00. ± 2.32	30.2± 1.10
2.0	0.0	0.0	0.0	0.0

On the other hand, the evaluation of photodynamic effect of phloxine B on growth of A. flavus and A. parasiticus and AFB1 production after exposure to solar simulator, reported that the mycelium dry weight (g/l) for both A. flavus and A. parasiticus decreased by increasing the concentration of phloxine B. At a concentration of (0.5 and 1 mg %) the mycelium dry weight was decreased to (112.0±3.00 g/l) and (60.3±1.93 g/l) for A. flavus and decreased to $(130.3\pm3.7 \text{ g/l})$ and $(96.0\pm2.92 \text{ g/l})$ for A. parasiticus, respectively. The complete inhibition of fungal growth occurred at a concentration of (2.0 mg %) phloxine B. AFB1 production decreased by increasing the concentration of phloxine B, at (1 mg%) concentration the AFB1 production was (27.33 µg/l) and (25.27 µg/l)for A. flavus and A. parasiticus, respectively. Whereas the AFB1 could not be detected at (2.0 mg%) phloxine B concentration for both A. flavus and A. parasiticus (Table, 4). However, photodynamic affect of phloxine B on growth of toxigenic strains of A. flavus and A. parasiticus and AFB₁ production after exposure to LED was studied.

The results revealed that the mycelium dry weight (g/l) and AFB₁ (µg/l) production for both *A. flavus* and *A. parasiticus* decreased by increasing the concentration of phloxine B. At a concentration of (0.5 and 1 mg%) the mycelium dry weight was decreased to (55.00 \pm 1.6 g/l) and (50.00. \pm 2.32 g/l) for *A. flavus*,and A.parasiticus, respectively. Whereas, complete inhibition of fungal growth occurred at a concentration of (1 and 2.0 mg%) phloxine B respectively. Also, AFB₁ production decreased by increasing the concentration of phloxine B, at (0.1 mg%) concentration. The AFB₁ production was (132.7 \pm 3.5 µg/l) and (125.3 \pm 2.35 µg/l) for *A. flavus* and *A. parasiticus*, respectively.

Whereas, the AFB₁ could not be detected at (2.0 mg%) phloxine B concentration for both *A. flavus* and *A. parasiticus* (Table, 5). These findings agree with the finding of **Friedberg et al.** (2001) who tested the fungicidal activity of the photosensitizers Green 2 w activated with 630 nm light against *A. fumigatus*. He found that the fungicidal activity of dye was both inoculums and light dose dependent. However **El- Adly** (2002) tested the photodynamic inactivation of seven isolates of dermatoplytes by different concentration of hematopropryin derivatives (HPD), methylene blue (MB) and toluidine blue O (TBO) after exposure to either solar simulator or natural sunlight.

The result showed significant growth inhibition when the solar simulator light was applied at rate of (400 w/m^2) . Luksiene et al. (2005) found that there is a clear correlation between the efficiency of inhibition of germination and the amount of photosensitizer accumulated by the fungus (dose dependent). The decrease in AFB₁ production may be due to either the fungicidal activity of phloxine B, which resulted in decreasing of fungal growth (mycelium weight) as there was a good correlation between mycelium weight and AFB₁ production or direct photo dynamic inactivation of AFB₁ by phloxine B or both. Also, Wilson and Mia (1993); Ouf and Abd Elhady (1999) found that incubation of C. albicans with toluidine blue or methylene blue as photosensitizer was necessary to render it susceptible to killing by laser light.

Also, **Abou Srea** (2005) found that He- Ne laser induced effects ranged from death of tested fungi to partial inhibition depending on the dye (crystal violet) concentration and the fungus under the test. The differences in responses to laser irradiation is usually attributed to specific pigmentation of irradiated propagates (**Antibus**, 1989). Therefore, it is believed that the difference in susceptibility of tested *Aspergillus* may be attributed to difference in melanin content, which may act as endogenous photosensitizer. This assumption is coupled with suggestion of (**Daub et al.** (1995) who stated that the difference in susceptibility of tested dermatophytes to photosensitization processes may be attributed to specific dark pigmentation, which may act as endogenous photosensitizer.

CONCLUSION

The presence of fungi and their toxins in feed and food reflected unhygienic measures during cultivation, irrigation harvesting transportation, handling, storage and processing of feed and food. Therefore, frequent testing programs of food during different stages of production must be monitored before given to animals or human for consumption. The fungal inhibitors may be added if the level of contamination over the limited level. Therefore, continuous investigations for finding new safe methods for controlling the growth of fungi and mycotoxins production are critical demand. The different methods of radiations particularly photodynamic inactivation is more applicable

method at large scale for degradation of AFB1 and control of fungal growth especially with solar simulator. All ways for increasing the quality of human health and animal's wealth.

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تاثير اشعة الشمس واشعة جاما واشعة الليزر على نمو فطريات الاسبرجيللس فلافس وافرازها سموم الافلاتوكسين.

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قد اشتملت هذه الدراسة على مائة (100) عينة من علائق الحيوان المصنعة و50عينة لبن من حالات التهاب الضرع و30عينة مسحات مهبلية من حالات اجهاض الاغنام والابقار وقد جُمعت هذه العينات من مزارع بمحافظة الجيزة من حيوانات مريضة تعانى من اعراض مرضية مختلفة تشمل الاسهال والاتهاب الرئوى فى العجول والابقار والاغنام والتهاب الضرع وبعض حالات الاجهاض.

وقد أكتشفت سموم الافلاتوكسين في 60% و60% من عينات العلائق والبان التهاب الضرع بمتوسط (110 ± 3.5 و 100 ± 3.5 و 100 ± 3.5 وقد تم در اسة تاثير تعرض العترات المعزولة من الاسبر جللس فلافس والاسبر جللس بار اسيتيكس وافراز سموم الافلاتوكسين ب 1 قبل وبعد التعرض لجر عات اشعة جاما والحث الضوئي لتقييم تأثير ها على نمو الفطريات وانتاج السموم . وقد اظهرت النتائج ان الجرعة 1 كيلوجراي من اشعة جاما كانت فعالة لمنح استنبات الجراثيم الفطرية والنمو الفطري لكلا من الاسبر جيلاس بار استيكس والاسبر جيلاس فلافس . حيث هبط انتاج سم الافلاتوكسين ب 1 عند الجرعات 1 كيلوجراي على التوالى. بينما كان استخدام ضوء يحاكي اشعة الشمس وضوء يحاكي اشعة الليزر في وجود مستحث ضوئي فلوكسين ب يؤدي الى تثبيط كامل لنمو الفطريات وانتاج سم الافلاتوكسين ب 1 عند جرعة 1 مجم 1 فلوكسين ب في حالة اشعة الشمس . على الجانب الاخرقد اتضح ان استخدام الليزر نتج عنه تثبيط كامل لنمو الفطريات وانتاج الافلاتوكسين ب 1 عند جرعات 1 عند جرعات 1 عند وقد نوقشت الاهمية الاقتصادية والصحية للنتائج الحالية.