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"Inactivation of Mycotoxigenic Molds in Rice (Oryza sativa) and Liquorice (Glycyrrhiza glabra) by Irradiation to Mitigate Mycotoxins Production"

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Abstract

Numerous studies have identified the mycotoxin contamination of rice and liquorice as a significant threat to human health in developing nations. Cereal grains and medicinal herbs can be highly infected by numerous fungus due to the variety of meteorological variables in this area as well as improper storage conditions, including high temperatures and humidity.Natural contaminants of different agricultural products with Aspergillus flavus, Aspergillus parasiticus, and Aspergillus ochraceus, as well as their mycotoxins, create health problems for humans and animals. The aim of this study is to investigate the effects of gamma irradiation doses on the growth of A. flavus, A. parasiticus, and A. ochraceus artificially inoculated in rice and liquorice, as well as on the production of their aflatoxins and ochratoxin A (OTA).It was found that irradiation doses of 2.0 and 4.0 KGy greatly reduced mycotoxin-producer molds and 6.0 KGy completely prevented molds growth. Irradiation dose of 4.0 KGy prevented the formation of aflatoxins, while 6.0 KGy prevented OTA production. Finally, we recommended studying the effect of radiation on health and life of experimental animals and then human health before consuming these irradiated foods.

Keywords*: Aspergillus*, Cereals, Gamma radiation, medicinal herbs, Mitigations, Mycotoxins

Introduction

Rice (*Oryza sativa* L.) grains are considered the most popular and important staple food in many countries worldwide and it is essential food in China and India ***(Satpathy et al., 2014; Gnanamanickam, 2009).*** Rice is also grown in Egypt and is one of the most popular foods. Approximately5.9 million tons of rice is produced in Egypt 2013(more than 22% of rice production in Africa)***(FAO, 2013).***

Liquorice (*Glycyrrhiza glabra* L.)is one of the important herbal medicines that grow in many countries ***(Miller, 1998).*** Liquorice root powder and extracts have known in Egypt from a long time. Liquorice root powder is famous for making soft drink and, confectionary and as sweeting and flavouring agent in food industry ***(Ariño, et al., 2007).*** It has also many applications in pharmaceuticals treatment like cough and to raise blood pressure ***(Fuhrman et al., 2002 andIsbrucker and Burdock, 2006).***

Rice grains and liquorice are susceptible to be contaminated with different mold species either pre-or post-harvest ***(Clavel and Brabet, 2013).*** Upon their favourable conditions of growth, some of these molds cause rotting and deterioration and others produce toxic substances (mycotoxins) leading to a great economic loss of these crops and cause serious health problems to humans ***(Richard, 2007).*** Aflatoxins that mainly produced by *A. flavus* and *A. parasiticus* are known to be the most potent carcinogenic agents among mycotoxins, and aflatoxin B1 is the most natural carcinogen (classified as Group 1 human carcinogen). Ochratoxin A, mainly produced by *A. ochraceus*, is the second important mycotoxins occurred mainly in grains, liquorice, coffee beans, paprika and black pepper, and is classified as 2B Group (possible human carcinogen) ***(IARC, 2009).***

For the high contamination of grains and herbal medicine plants with spoilage and pathogenic microorganisms including mycotoxigenic molds, it is highly needed to use a novel and non-thermal technology for decontamination of harmful microorganisms to improve the quality and safety of these commodities. It has been proven that food irradiation technology is very effective and sufficient in decontamination of harmful microorganisms without or with minimum changes in the quality and nutritional value of the treated products ***(Jafarzadeh et al., 2022 andSchmidt et al., 2019).***

Food irradiation technology is a physical-cold process for food preservation and only alternative to toxic fumigants and harmful food additives that usually used forpreventingdevelopment of microorganism contaminating foods as well as many other purposes. It is approved by national, regional and international authorities and organizations as a safe and environmental friendly ***(da Silva Aquino, 2012).***Food irradiation technology is achieved with only application of gamma radiation, electron beams or X-rays. Special labelling (**Radura symbol**), **Fig. 1** is required to indicate that this food is irradiated ***(Morehouse and Komolprasert, 2004).*** However, due to the negative impact of this symbol on the consumers many food industries use alternative phrase such as "electronically pasteurized" ***(Fox, 2002).***

The main objectives of this study are

Isolation and identification of molds occurring on rice and liquorice, screening for mycotoxins production and determination of the radio-sensitivity of the isolated mycotoxigenic molds. In the same time, inactivation of these molds by using gamma irradiation to mitigate mycotoxin production was also studied.

Materials and Methods

Collection of samples:

Ten husked Egyptian rice samples and seven liquorice dried samples were collected from different markets at Egypt. The samples were packaged (50gm of each) in poly ethylene bags.

Isolation and purification of molds:

Each package of rice grainsand liquorice were handled under aseptic conditions after being wiped with ethanol (70%) wetted cotton for surface sterilization. Ninety ml of sterilized physiological saline solution (0.85 % NaCl) were added to 10 gm of each sample, and homogenized for 2 min using Stomacher (Laboratory Blender, STOMACHER 400). Serial dilutions were prepared with the same saline solution. Czapek's Yeast Agar (CYA) medium was added to the center of sterile petri dishes after 1ml of each dilution was placed there. The plates were incubated at 28°C±1 for 3-5 days after solidification; the appeared mold colonies were isolated and purified. The purified isolates were transferred to CYA slants and kept at 2ºC±1 for further investigation.

Identification of molds

One hundred and seventeen isolateswere isolated from rice, as well as 42 isolates isolated from liquorice and all were identified according to their morphological characters at CYA medium and microscopically confirmed at Assiut University- Moubasher Mycological center (AUMMC), Faculty of Science.

Preparation of mold spore suspension:

Each isolate of *A. flavus, A. parasiticus,* and *A. ochraceus* from stock CYA slants was grown on the surface of a 250 ml-conical flask containing 100 ml of CYA medium (5 flasks were used). To promote sporulation, the inoculated flasks were incubated at 28°C±1 for 14 days. Mold spores were harvested by adding 100 mL of

sterilized Tween-80 solution (as a surfactant), vortexing for 5 minutes, filtering through several layers of sterilized chess cloth, and centrifuging (10000 rpm) for 15 minutes. The supernatant was collected in a 250 ml sterilized conical flask. After washing the centrifuge tubes three times with distilled water, the suspension was transferred to the 250 ml conical flask and re-suspended with Tween-80 solution.

Cultivated aflatoxins production and ochratoxin A:

Two ml of spore suspension of each one of the seventeen *Aspergillus flavus* and the two *Aspergillus parasiticus*isolates which isolated from rice samples and sixteen *A. flavus* isolateswhich isolated from liquorice samples were added to 250-ml conical flask containing 50 ml of CYB medium. The inoculated flasks were incubated at 28ºC±1 for 10 days. Eight *A. ochraceus* isolates which isolated from rice and 6 isolates which isolated from liquorice were inoculated in 50ml of yeast extract sucrose (YES) broth (yeast extract 40.0 gm, and 20.0gm) pH was adjusted at 6.5 following sterilization in a 250-ml conical flask and incubated at 28ºC±1 for fungal growth and toxin synthesis after being incubated for 10 days.

Extraction of aflatoxins from liquid medium:

To kill mold spores and ease the extraction of aflatoxin, the incubated flasks containing *A. flavus* spore suspension were autoclaved at 100°C for 30 seconds ***(Tsai et al., 1984)***. Then, aflatoxins were extracted from the culture (mycelium and broth medium) by adding 3-fold chloroform and vigorously shaking the mixture for 30 minutes using a shaker (Precise Shaking Incubator, Model: WIS-10R, Korea) ***(AOAC, 2000).*** The mixture was filtered over anhydrous sodium sulphate using filter paper. The filtrate was separated using separating funnels. Aflatoxin-containing chloroform extract was evaporated with a rotary evaporator to almost dryness (1 ml).

Extraction of ochratoxin A from liquid medium:

Ochratoxin A was extracted from the culture (mycelium and broth medium) with adding acetone: water (70:30 v/v) ***(AOAC, 2000),*** shaking well for 30 min using shaker (Precise Shaking Incubator, Model: WIS-10R, Korea). After well shaking, the mixtures were filtrated through filter paper over anhydrous sodium sulphate. The filtrate was separated using separating funnels.The extraction containing ochratoxin A was evaporated to near dryness (1 ml) using rotary evaporator.

Detection of aflatoxins and ochratoxin A using Thin layer chromatography (TLC):

Aflatoxins and ochratoxin A were discovered TLC usingpre-coated glass plates (20x20 cm) with a thin layer (0.25 mm) of silica gel 60 GF_{254}. Aflatoxins (B1, B2, G1, and G2) or ochratoxin A standard work solution (10 µL / ml in chloroform) and 40 aliquots of each sample extracts (aflatoxins or ochratoxin A) were independently spotted on the TLC plates. In the case of aflatoxins, the spotted plates were generated in a chromatographic jar with a running solvent ratio of 9:1 chloroform to acetone ***(Ramesh et al., 2013).*** In the instance of ochratoxin, the spotted plates were developed in a chromatographic jar using a flowing solvent mixture of toluene, ethyl acetate, and formic acid 88% (6:3:1) ***(Santos and Vargas, 2002).***

Artificial inoculation of husked rice with *A. flavus* and *A. parasiticus* and liquorice with *A. ochraceus*:

To eliminate natural microflora, half kilograms of husked rice and liquorice samples were radiation-decontaminated (15KGy).

Under aseptic conditions, the moisture content of these samples was increased to 15-18% using sterilized distilled water to promote mold growth and mycotoxin production. Under aseptic condition, 25gmof each sample were inoculated with 2ml of the previously prepared spore suspension of each mold (individually). The inoculated samples of each mold were derived into four groups;

the first, second, and third groups subjected to 2.0, 4.0, and 6.0 KGy of gamma radiation, respectively, while the fourth group was left un-irradiated as a control.

Irradiation process:

Irradiation processwas carried out at the National Center for Radiation Research and Technology (NCRRT), Nasr city, Cairo, Egypt, using a cobat-60 irradiator source (Indian Gamma Cell). At the time of irradiation, the dose rated of the irradiator was 0.797 KGy/h during irradiation processes. Alanine dosimeters (Traceable to National Physical Laboratory, UK) were used for dose calibration of the irradiator and for measuring the average absorbed dose. Detailed dose mapping was conducted by Department of Radiation Protection and Dosimeter according to Egyptian Standards.

Storage:

All irradiated and non-irradiated husked rice and liquorice samples were stored at ambient temperature (25-30°C) for 60 days. At intervals of 15 days, the mold counts were determined. Aflatoxins that produced in husked rice and OTA that produced in liquorice were quantitatively estimated after 30 days of storage using HPLC.

Determination of D_{10}- value for the highly mycotoxins producers:

The radiation sensitivity or resistance of a microbe is measured by its D_{10}-value. Two and half ml of each *A. flavus, A. parasiticus*, and *A. ochraceus* spore-suspension 10^6–10^7 (cfu/ml) were added to 25gm of radiation decontaminated (15 KGy) rice and liquorice, respectively in polyethylene bags. Three replicates were employed for each dose of gamma irradiation applied to inoculated bags (0.0, 0.5, 1.0, 1.5, 2.0, and 3.0 KGy). The number of colonies that survived after each dosage of radiation was measured afterward. The radiation dose-response curve for each fungus was constructed by plotting the survival counts against irradiation dose. The slope of

the curves was used to estimate the D_{10}-values, which were then calculated using the following equation:

$D_{10}={}^{-1}/_{b}$

$$b = \frac{\sum xy - n\bar{x}\bar{y}}{\sum x^2 - n\bar{x}^2}$$

x = Dose level (KGy)

y = Log number of bacterial surviving after receiving x amount of radiation

n = Number of calculated point

Quantitative estimation of aflatoxins and ochratoxin A using HPLC:

Reagents and chemicals:

Aflatoxins standards (AFB1, AFB2, AFG1 and AFG2), OTA and trifluoro-acetic acid (TFA) were purchased from Sigma-Aldrich (St. Louis, Mo, USA). Methanol, diethyl ether anhydrous, acetonitrile, chloroform and hexane of HPLC grade solvents were obtained from (ROTH, Germany). Silica gel (60-120, mesh)for column chromatography were obtained from (SDFCL, India).Silica gel60 GF_{254} for TLC was obtained from ADVENT, India. Deionized water generated from (Thermal Electron LED GmbH, Sweden) was used.

Calibration standards:

Aflatoxin stock solutions (10 µl/ ml) were prepared by dissolving each aflatoxins or ochratoxin Ain 5 ml of methanol. Working solutions (0.25, 0.5, 1.0, 5, 10, 25 and 50 ng/ml) were prepared to established the calibration curve. The derivatization of aflatoxins standard was performed by the addition of 50µL of TFA and 200 ml of n-hexane and vortexing the mix for 30 seconds, the derivatization of ochratoxin A standard was performed by addition of 300 µl of OTA extract was mixed with 300 µl of boron trifluoride (BF_3)

solution. The mixture was heated at 70ºC for 20 min and used directly for HPLC analysis.

Aflatoxins and ochratoxin A were quantitatively determined by using HPLC (Agilent 1260 series) equipped with a quaternary pump (G1311C), auto sampler (G1329BA), micro-vacuum degasser (G33223), and fluorescence detector (G1321C).

The chromatographic column used throughout the injections was Zorbax Eclipse XDB-C18 (50 mmx 4.6 mm ID; 1.8 µm partial size) Integrated Column Compartment (ICC) (G7129A). The HPLC mobile phase for aflatoxin analysis was composed of deionized water, methanol, and acetonitrile (50:25:25 v/v/v) with the flow rate adjusted to 1 ml/min; fluorescence detector conditions for emission of 360 nm, For OTA analysis, the HPLC composition contained acetonitrile and methanol (60:40 v/v) with a flow rate of 1ml/min and fluorescence detector conditions of 220 nm for emission.

Sample preparation

According to the ***AOAC,2000,17thed*.** standard test method ***AOAC 17th, 977.16*** for aflatoxins and ***AOAC, 973. 37*** for OTA,fifty grams of each sample contaminated with aflatoxins were extracted, then dried to 50ºC for 24 hr. and grinded it to fine powder at maximum particle size reduction and the thoroughness of mixing to achieve effective distribution of contaminated portions. The fine powder was mixed with water: chloroform (25:150 v/v); the mixture was shaked for 60 min/200 rpm mechanical shaker and then filtered through double thickness filter paper Whatman no. 1 overanhydrous sodium sulphate, received the filtrate to 500 ml separation funnel, the upper clear layer transferred to cleaned conical flask then evaporated by using rotary vacuum evaporatorto reach about 50 ml. The chloroform extract was purified and cleaned up by hexane and anhydrous diethyl ether through silica gel column chromatography. The aflatoxins were eluted by chloroform : methanol (97:3 v/v). The

eluent was evaporated to near dryness (1-2 ml) by using rotary vacuum evaporator, and derivatization was conducted to be identical to the calibration standard; 25 µl was then injected into the HPLC.The same procedure was followed with OTA except the derivatization was done with BF_3 that mentioned before.

Statistical analysis:

All data were swift as the mean ± SD (standard division) of three replicates. The significance of the data with different factors was evaluated using one-way and two way analysis of variance ANOVA, and Dunken test at a reliability level was used to identify different between each treatment level. All analyses were performed with SAS software package version 9.0 TS Mo (SAS Institute Inc., Cary, NC, USA) (**SAS, 2002**).

Results and Discussion

Isolation of molds:

A total of 117 mold isolates were isolated from the ten collected samples of husked rice. The isolated mold species belonged to seven genera namely, *Aspergillus* (62 isolates), *Penicillium* (42 isolates), *Fusarium* (4 isolates), *Rhizopus* (3 isolates), *Cladosporium* (3 isolates), *Altrernaria* (2 isolates) and *Emericella*(1 isolate), as tabulated in **Table 1**. *Aspergillus* and *Penicillium* species were predominant. *Aspergillus* consisting of 10 species and *Penicillium* consisting of 13 species. *A. flavus* and *A. niger* were the most predominant among Aspergilli followed by *A. ochraceus*. *P. expansum* and *P. crustosum* were the most predominant among Penicilli followed by *P. italicum*.Many other investigators have been reported similar mold genera and species in rice. ***Hafez, et al., (2004)*** isolated and identified many mold species from paddy rice.They were *A. flavus*, *A. ochraceus*, *A. fumigantus*, *A. terrus*, *A. sydowii*, *P. chrysogenum*, *P. corylophilum*, *F. oxysporum*,

Cladosporiumcladosporloides, *Alternariatenus*, *Trrichodermaviridi* and *Mucorraemosus*. ***Reddy et al., (2006)*** reported that *A. flavus*, *A. parasiticus* and A. ochraceus were the most predominant mold species in rice grains. ***Reddy, et al., (2008)*** reported that mold species of the genera *Aspergillus*, *Penicillium* and *Fusarium* were the main mycotoxigenic molds found in rice. Several species of mold have been also found in rice, including *A. flavus*, *A. ochraceus*, *A. versicolor*, *A. niger*, *P. citrinum*, *P. verrucosum, P. islandicum, F. graminearum, F. proliferatum, Alternaria alternate* and *Cladosporiumcladosporloides* ***(Abbas et al., 1999; Oerke and Dehne, 2004; Reddy et al., 2004; Makun et al., 2007; Gonçalves et al., 2019).*** According to our results and the reported findings, it is clear that the mold genera and species were different might be due to the rice variety and the location of growing.

Table 2 shows the main mold genera and species which isolated from liquorice collected samples. It is obvious that six genera were identified, they were *Aspergillus* (38 isolates), *Penicillium* (23 isolates), *Rhizopus* (3 isolates), *Eurotium* (4 isolates), *Alternaria* (5 isolates) and *Ulocladium* (1 isolate).*A. flavus* followed by *A. niger* and *A. ochraceus* were abundant among Aspergilli and *P. chrgsogenum* was abundant among Penicilli.

Chen et al., (2013) isolated and identified 22 mold species belonging to genera from fresh and dry liquorice. They reported that Aspergillus and Penicillium were the most two genera abundant in both fresh and dry liquorice. **Chen et al., (2011)** isolated and identified 16 mold species affiliated to Aspergillus, Penicillium and Eurotium from different liquorice root samples.**Toma and Abdulla (2013)** Isolated and identified ten different fungal genera and 16 species from spices and medicinal plantsas Alternariaalternata, Aspergillus spp., Gliocladium sp., Hyalodendrondiddeus, Memmoniella sp., Penicillium spp., Rhizopus spp., Syncephalastrum sp., Cladosporiumlignicolum and Ulocladium botrytis. Spices and

herbs are among the food products that are most commonly contaminated with toxigenic molds and mycotoxins due to their processing steps (harvesting methods, drying, storage), environmental factors, and environmental conditions, particularly aflatoxins and aflatoxigenic fungus ***(Ozbey and Kabak, 2012;El-Mahgubi et al., 2013; Hammami et al., 2014).***

Screening for aflatoxins production from isolated fungi strains:

The seventeen *A. flavus* isolates and the two *A. parasiticus* isolates which isolated from rice were tested for the production of aflatoxins in Capek's Yeast Extract liquid medium using TLC method. **Table 3** revealed that out of 17 *A. flavus* isolates, six isolates (35.3%) produced aflatoxin B1 (AFB1) and aflatoxin B2(AFB2) but at different levels. None of these isolates produced aflatoxin G group. The two *A. parasiticus* isolates were also tested for aflatoxins production. *A. parasiticus*isolate (no.18**) was found to produce both AFB1 and AFG2, while the other isolate (no.19**) did not produce any of aflatoxins. *A. flavus* and *A. parasiticus* have been reported by many investigators to be the most producers of aflatoxins ***(Rodriguez-Gilet al., 2007; Udomkun et al., 2017).*** It has been reported that *A. flavus* produces only aflatoxin B group (AFB1 and AFB2), while *A. parasiticus* produce both aflatoxin B group and G group (AFB1, AFB2, AFG1 and AFG2) as reported by many researchers ***(Fazekas et al., 2005; Novas and Cabral, 2002).*** Many other investigators reported aflatoxin production by *A. flavus* and *A. parasiticus* in synthetic liquid medium and in grains like wheat, corn, rice, sorghum, oat, etc. ***(Diener and Davis, 1969; Kumaret al., 2008; Neme and Mohammed, 2017).***

Sixteen *A. flavus* isolates which were isolated from liquorice were also screened for aflatoxins production in liquid medium. Out of the 16 isolates, 4 isolates (25%) were found to produce AFB1 and AFB2 **(Table 4)**. None of these isolates produced aflatoxins G group.***Azzoune,et al.(2015)*** isolated many mold species from (44)

different spices samples. The species were *Aspergillus* (56.4%), *Penicillium* (25.1%), *Mucor* (12.8%) and *Eurotium* (5.7%). Species belonging to *Aspergillus* section flavirepresented 28.9% of the total Aspergilli.Ninety-four isolates of 245 *Aspergillus* section flavifrom examined produced aflatoxins. The most frequent chemotypes (84%) correspond to isolates able to produce both aflatoxin B and cyclopiazonic acid followed by the producers of only aflatoxin B. Twenty-three (63.9%) of the 36 spices contained AFB1.

Screening for ochratoxin A production from isolated fungi strain:

The eight *A. ochraceus* isolates which isolated from the collected rice samples and 6 isolates which isolated from collected liquorice samples were tested for their ability to produce OTA in YES broth medium.

Out of the 8 *A. ochraceus* isolates isolated from rice samples, 2 isolates (25%) were found to produce OTA. Out of 6 A. ochraceus isolates isolated from liquorice, 2 isolates (33.3%) produced OTA (**Table 5**). It has been reported that many species of *Aspergillus* can produce OTA such as*A. ochraceus,A. carbonarisA. alliaceus, A. melleus, A. sulphurus*and *A. niger* ***(Kontaxakis, 2013; Gupta et al., 2018;Taghizadeh et al.,2018).***

The most common mold producing OTA in agricultural commodities is *A. ochraceus* ***(Khalil et al., 2021)***.Ochratoxin A as a metabolite of *Aspergillus ochraceus*, it has since been discovered in a wide range of agricultural products from different geographical areas of the world ***(Pardo et al., 2004; Adeyeye, 2016).***

Radiation sensitivity of *A. flavus*, *A. parasiticus and A. ochraceus*:

The radiation sensitivity of the highly aflatoxins producers, i.e*A. flavus* (isolate No. 13) and *A. parasiticus*(isolate No. 18**) were measured by the determination of the D_{10}-value from the radiation

dose response curve. D_{10}-value of a microbe is defined as the radiation dose (KGy) required to reduce the population of that microbe by 10-fold (by one log cycle), i.e required to kill 90% of the total number. The radiation dose-response curve is constructed by plotting irradiation dose against log survival counts.**Tables (6,7)** and **Figures(2,3)** show that irradiation doses used greatly reduced the viable counts of *A. flavus* and *A. parasiticus* and the reduction was parallel to increasing irradiation dose. Irradiation dose of 3.0 KGy reduced the counts of these molds to below detectable level (<10cfu/g). ***Maity et al., (2011)*** found that irradiation dose of 3.0 KGy inactivated *A. flavus* which isolated from rice.

The calculated D_{10} values of*A. flavus* and *A. parasiticus* in rice were 0.540 and 0.513 KGy, respectively. Similar results have been found: ***Ragab et al. (1994)*** determined the D_{10}-values of the two aflatoxin- producing molds, namely *A.flavus* and *A. flavus forming sclerotia* in synthetic liquid medium and in sesame seeds. They found that their D_{10}-values were 0.53 and 0.6 KGy and 0.53 and 0.56 KGy in liquid medium and in sesame seeds, respectively. ***Aziz et al., (2004)*** found that the D_{10}-value of *A.flavus* isolated from spices was 0.52 KGy. ***Abdel-Khalek (2008)*** found that the D_{10}-values of *A.flavus* in cumin and in caraway were 0.59 and 0.45 KGy, respectively indicating that the D_{10}-value of the same fungus differs according to the matrix.

Table (8) and **Fig. 4** indicate that as irradiation dose increased, the count of *A. ochraceus* decrease in proportional trend. The calculated D_{10}-value in liquorice was 0.454 KGy. It is obvious from these results that the radio-sensitivity was different among the studied molds;i.e differed from mold species to another in the same genus. This could be due to the strain within the species, source of isolates and matrix ***(Schmidt et al., 2018).Aziz et al., (1997)*** reported thatthe calculated D_{10}-values for *A. ochraceus* and *A. parasiticus*, were 0.36 and 0.48 KGy, respectively**.**

Effect of gamma irradiation on the total counts of *A. flavus* and *A. parasiticus* inoculated into rice with moisture content of 15-18%:

The effect of gamma irradiation at levels of 2.0, 4.0 and 6.0 KGy on the production of aflatoxins produced by*A. flavus* and *A. parasiticus* inoculated into rice samples (moisture content of 15-18%) are tabulated in **Table (9), and Figure (5).**The initial log count of *A. flavus* and *A. parasiticus* together artificially inoculated in control rice samples at zero time was 6.17. Irradiation doses of 2.0 and 4.0 KGy reduced this count by 1.33 log (21.56%) and 5.05 log (81.8%), respectively. Meanwhile, irradiation doses of 6.0 KGy completely destroyed the cells of these fungi as their log count was below the detectable level. During storage, the counts of *A. flavus* and *A. parasiticus* in inoculated control samples increased from 6.17 to 8.0 logs after 60 days of storage.

The counts of these fungi in 2.0 and 4.0 KGy irradiated samples also increased from 4.84 to 6.69 logs and from 1.12 to 2.9 logs, respectively. The counts of *A. flavus* and *A. parasiticus* in rice samples exposed to 6.0 KGy were below detectable level throughout storage period.

Effect of gamma irradiation on the aflatoxins produced by *A. flavus* and *A. parasiticus:*

Table (10)shows the results of aflatoxins as determined by HPLC after 30 days of storage. It is clear that the concentrations of AFB1, AFB2 and AFG2 in rice samples (15-18% moisture content) after 30 days of storage were 32.1, 15 and 224.1 µg/Kg, respectively.

AFB1 and AFG2 were only discovered in rice samples irradiated at 2.0 KGy but at lower levels, i. e. 26.4 and 22.8 µg/Kg, respectively. This indicate that this irradiation dose greatly reduced the formation of aflatoxins might be due to the great reduction in the aflatoxins-producers molds (as indicated in Table 9), but still more than the accepted limits seated by many efforts. The most frequently

limit for total aflatoxins is at 4*µ*g/Kg which applied by more countries and for AFB1 is at 2*µ*g/Kg ***(EC, 2010).***

Codex Alimentarius established a level of 15 *µ*g/Kg aflatoxin total in almonds, hazelnuts and pistachios intended for further processing and a level of 10 *µ*g/Kg aflatoxin total in almonds, hazelnut and pistachios ready-to-eat. According to the national standard of Iran, the maximum residue level (MRL) for total aflatoxins and AFB1 in rice have been reported to be 30 and 5 *µ*g/Kg, respectively ***(ISIRI,2002).***

Although irradiation dose of 4.0 KGy did not completely prevented the growth of these mold species, it was completely prevented the formation of aflatoxins by the survival colonies. Irradiation dose of 6.0 KGy completely eradicate *A. flavus* and *A. parasiticus* in rice and subsequently no aflatoxins were produced. Our results are in agreements with those reported by***Khalil et al. (2021)*** who found that irradiation dose of 4.5 KGy reduced the production of aflatoxins and ochratoxin A in yellow maize.

Gamma irradiation of 6.0 KGy reduced the mold counts of red chillies by 5 logs, as well as it reduced the AFB1 and total aflatoxins in ground and whole chillies from 35 and 39 µg/kg in non-irradiated control samples to 18 (94.8%) and 4.0 (89.7%) µg/kg, respectively. Natural contamination of agriculture commodities with mycotoxins linked with the growth of mycotoxigenic molds, thus inactivation of these mycotoxigenic molds in food products make these products free from mycotoxins and hence protect consumer's health. Early in (1989)IAEA reported that 2.5 to 6.0 KGy irradiation doses are required to prevent mold growth in agriculture products, hence no mycotoxins are found ***(Albuzaudiet al., 2017).***

Effect of gamma irradiation on the total counts of *A. ochraceus* inoculated into liquorice with moisture content of 15-18%:

The effect of gamma irradiation at levels of 2.0, 4.0 and 6.0 KGy on the production on the log count of*A. ochraceus* inoculated in liquorice (moisture content of 15-18%) is tabulated in **Table (11), and Figure (6).**

The initial log count of *A. ochraceus* artificially inoculated in control liquorice samples at zero time was 5.43. Irradiation doses of 2.0 and 4.0 KGy reduced this count by 1.61 log (29.69%) and 4.27 log (78.64%), respectively. Meanwhile, irradiation doses of 6.0 KGy completely destroyed the cells of these fungi as their log count was below the detectable level. During storage, the counts of *A. ochraceus* in inoculated control samples increased from 5.43 to 7.69 logs after 60 days of storage. The counts of these fungi in 2.0 and 4.0

KGy irradiated samples also increased but at lower levels, i. e. from 3.82 to 6.21 logs and from 1.16 to 2.64 logs, respectively. The counts of *A. ochraceus* in liquorice samples exposed to 6.0 KGy were below detectable level throughout storage period.

Effect of gamma irradiation on the ochratoxin A produced by *A.ochraceus*:

Table 12 indicates that *A. ochraceus* produce ochratoxin A in non-irradiated control samples after 30 days of storage at ambient temperature (25-30^{o}C), the concentration of these OTA as determined by the HPLC was 7800 µg/Kg in liquorice indicating the highest concentration in non-irradiated samples. Irradiation dose of 2.0 KGy reduced the production of OTA by 56.41%, and 4.0 KGy irradiation dose reduced OTA production by 95.38%. *A. ochraceus* in liquorice was completely prevented by a 6.0 KGy irradiation dose, and consequently no OTA was therefore formed. Irradiation dose of 4.0 KGy did not completely prevented A. ochraceus growth, but it

was completely prevented the formation of ochratoxin A by the survival colonies.

The European commission (EC) Regulation No.165/2010 set maximum levels for OTA in unprocessed cereals of 5.0 µg/Kg. The maximum level for all products derived from processed cereals including processed cereals products and cereal intended for direct human consumption is at 3.0 µg/Kg. The same fixed maximum levels of OTA of 20 and 80µg/Kg for liquorice root and for liquorice extract, respectively ***(EC, 2010).***

Ariño, et al., (2007) reported that the average amount of OTA in the dry licorice root samples was 63.6 ng/g, with concentrations ranging from 1.4 to 252.8 ng/g. A chromatogram of a dry licorice root sample containing ochratoxin at a level of 111.9 ng/g. ***Marinet al., (2011)*** Mycotoxins are estimated to contaminate 25% of agricultural crops worldwide, according to the FAO. They also have a big impact on the economy because they result in losses in farm animals, making it more difficult to manage them, or make commodities unusable for national or international trade because they don't follow the rules (**EC, 1881/2006, 105/2010, and 165/2010**).

Table (1). Incidence of mold genera and species isolated from rice samples:

Isolated Molds genera	No. of isolates	Percentage of total number	
Aspergillus	62	% from Aspergillus	% of total Number(117)
A. flavusvarcolumnaris	3	4.84	2.56
A. flavus	11	17.74	9.40
A. flavus forming sclerotia	3	4.84	2.56
A. parasiticus	2	3.23	1.71
A. terreus	4	6.45	3.42
A. nidulans	2	3.23	1.71
A. ustus	9	14.52	7.69
A. ochraceus	8	12.90	6.84
A. niger	13	20.97	11.11
A. sydowii	2	3.23	1.71
A.melleus	2	3.23	1.71
A. terricola	2	3.23	1.71
A. fumigantus Fresenius	1	1.61	0.85
Penicillium	42	% from Penicillium	% of total Number(117)
P. expasum	7	16.66	5.98
P. verrucosum	5	11.90	4.27
P. echinulatum	5	11.90	4.27
P. italicum	4	9.52	3.42
P. waksmanii	2	4.76	1.71
P. griseofulvum	2	4.76	1.71
P. crustosumthom	7	16.67	5.98
P. chrysogenium	3	7.14	2.56
P. islandicus	1	2.38	0.85
P. duclauxii	2	4.76	1.71
P. assiutensis	1	2.38	0.85
P. flavus	2	4.76	1.71
P. sclerotiorum	1	2.38	0.85
Fusarium spp.	4		3.42
Rhizopus spp.	3		2.56
Cladosporium spp.	3		2.56
Alternaria spp.	2		1.71
Emericellavoriecolor	1		0.85

Table (2). Incidence of mold genera and species isolated from Liquorice samples:

Isolated Molds	No. of isolates	Percentage of total number	
Aspergillus	38	% from Aspergillus	% of total Number(74)
A. flavus	16	69.57	21.62
A. terreus Thom	4	17.39	5.40
A. ochraceus	6	26.09	8.10
A. niger	9	39.13	12.16
A. terricola	1	4.34	1.35
A. A. fumigantus Fresenius	2	8.70	2.70
Penicillium	23	% from Penicillium	% of total Number(74)
P. crustosum Thom	4	17.39	5.40
P. verrucosum	4	17.39	5.40
P. oxilicum	2	8.70	2.70
P. echinulatum	2	8.70	2.70
P. italicum	2	8.70	2.70
P. islandicus	2	8.70	2.70
P. citrinum Thom	2	8.70	2.70
P. chrysogenum	5	21.74	6.76
Alternariaspp.	5		6.76
Eurotium spp.	4		5.40
Rhizopus spp.	3		4.05
Ulocladium chartarum	1		1.35

Table (3). Screening for aflatoxins production by *A. flavus* and *A. parasiticus* isolates which isolated from rice in Czapek's liquid medium using TLC:

Isolate No.	AFB1	AFB2	AFG1	AFG2
1	-	-	-	-
2	+	+	-	-
3	-	-	-	-
4	++	++	-	-
5	-	-	-	-
6	-	-	-	-
7	-	-	-	-
8	+	+	-	-
9	-	-	-	-
10	-	-	-	-
11	++	++	-	-
12	-	-	-	-
13	+++	++	-	-
14	-	-	-	-
15	-	-	-	-
16	-	-	-	-
17	++	++	-	-
18**	++	-	-	++++
19**	-	-	-	-

(+++) strong production, (++) Moderate production, (+) Weak production, (-) No production

**: refers to the two isolated identified as *A. parasiticus*

Table (4). Screening for aflatoxins production by *A. flavus* isolates isolated from liquorice in Czapek's liquid medium using TLC:

Isolate No.	AFB1	AFB2	AFG1	AFG2
1\	-	-	-	-
2\	-	-	-	-
3\	+++	+++	-	-
4\	-	-	-	-
5\	-	-	-	-
6\	-	-	-	-
7\	-	-	-	-
8\	-	-	-	-
9\	-	-	-	-
10\	-	-	-	-
11\	-	-	-	-
12\	++	++	-	-
13\	+	+	-	-
14\	-	-	-	-
15\	++	++	-	-
16\	-	-	-	-

(+++) strong production, (++) Moderate production, (+) Weak production,
(-) No production

Table (5). Screening for OTA production by *A. ochraceus* isolates in YES broth medium using TLC:

Rice		Liquorice	
Isolate No.	OTA	Isolate No.	OTA
7	-	27\	-
9	-	30\	++
16	++	33\	-
59	-	34\	+++
64	-	35\	-
66	-	36\	-
89	-		
102	++		

(+++) strong production, (++) Moderate production, (+) Weak production,
(-) No production

Table (6). Effect of incremental gamma radiation dose on the counts of *Aspergillus flavus* isolated from rice and found to produce AFB1 and AFB2:

Irradiation dose (KGy)	(cfu/g)	Log cfu/g
0.0	$5.3x10^5$	5.724
0.5	$6.0x10^4$	4.78
1.0	$1.3x10^3$	3.11
1.5	$7.0x10^2$	2.85
2.0	$8.3x10^1$	1.92
3.0	<10	0

Table (7). Effect of incremental gamma radiation dose on the counts of Aspergillus *parasiticus* isolated from rice and found to produce AFB1, AFB2, AFG1 and AFG2:

Irradiation dose (KGy)	(cfu/g)	Log cfu/g
0.0	$2.9x10^5$	5.46
0.5	$8.7x10^4$	4.94
1.0	$1.8x10^3$	3.26
1.5	$4.3x10^2$	2.63
2.0	$1.0x10^1$	1.0
3.0	<10	0

Table (8). Effect of incremental gamma radiation dose on the counts of Aspergillus ochraceus isolated from rice and found to produce OTA:

Irradiation dose (KGy)	(cfu/g)	Log cfu/g
0.0	$2.4x10^6$	6.38
0.5	$1.33x10^5$	5.12
1.0	$1.0x10^4$	4.0
1.5	$5.0x10^2$	2.70
2.0	$1.7x10^1$	1.23
3.0	<10	0

Table (9). Effect of gamma irradiation and storage at ambient temperature on the total counts of A. flavus and A. parasiticus inoculated into rice:

Storage period (Days)	Irradiation Doses (KGy)			
	0.0	2.0	4.0	6.0
Zero time	6.17^{a}_{d} ±0.08	4.84^{b}_{d}±0.01	1.12^{c}_{d}±0.10	ND
15	6.10^{a}_{c}±0.04	4.66^{b}_{c}±0.11	1.78^{c}_{c}±0.09	ND
30	7.47^{a}_{b}±0.00	5.30^{b}_{b}±0.02	2.61^{c}_{b}±0.13	ND
60	8.0^{a}_{a}±0.00	6.69^{b}_{a}±0.006	2.90^{c}_{a}±0.05	ND

* ND: Below detectable level (<10 cfu/g)

Mean values followed by different superscript (within rows) and different superscripts (within columns) are significantly different ($\rho < 0.05$)

Table (10). Effects of gamma irradiation on the reduction of pre-formed aflatoxins in Rice

Irradiation doses (KGy)	Aflatoxins (µg/Kg)			
	B1	B2	G1	G2
Control	32.1	15	ND	224.1
2.0	26.4	ND	ND	22.8
4.0	ND	ND	ND	ND
6.0	ND	ND	ND	ND

Table (11). Effect of gamma irradiation and storage at ambient temperature on the total counts of *A. ochraceus* inoculated into liquorice:

Storage period (Days)	Irradiation Doses (KGy)			
	0.0	2.0	4.0	6.0
Zero time	5.43^{a}_{d} ±0.07	3.82^{b}_{d}±0.10	1.16^{c}_{d}±0.15	ND
15	5.75^{a}_{c}±0.03	4.44^{b}_{c}±0.12	1.39^{c}_{c}±0.21	ND
30	6.94^{a}_{b}±0.05	4.47^{b}_{b}±0.07	2.35^{c}_{b}±0.16	ND
60	7.69a$^{a}_{a}$±0.01	6.21^{b}_{a}±0.06	2.64^{c}_{a}±0.14	ND

*ND: Below detectable level (<10 cfu/g)

Mean values followed by different superscript (within rows) and different superscripts (within columns) are significantly different ($\rho < 0.05$).

Table 12. Effects of gamma irradiation on the production of ochratoxin A in artificially inoculated liquorice with *A. ochraceus* (after 30 days of inoculation 28°C± 1)

Liquorice	Ochratoxin A (µg/kg) Irradiated dose (KGy)			
Storage period (Days)	Control	2.0	4.0	6.0
30 day	7800	3400	360	ND

Fig 1. International Radura.

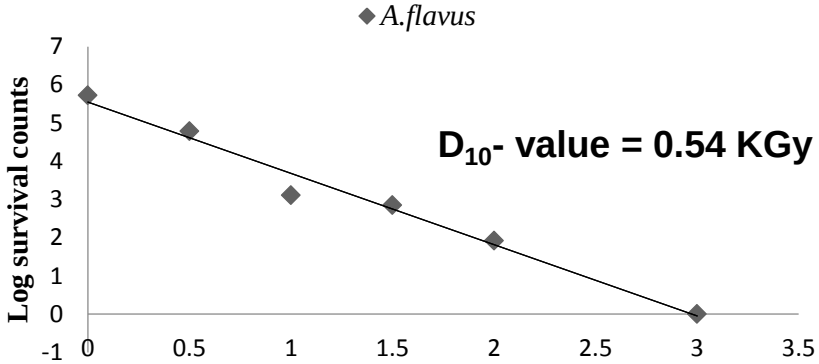

Figure (2); Radiation dose-response curve of *A. flavus*

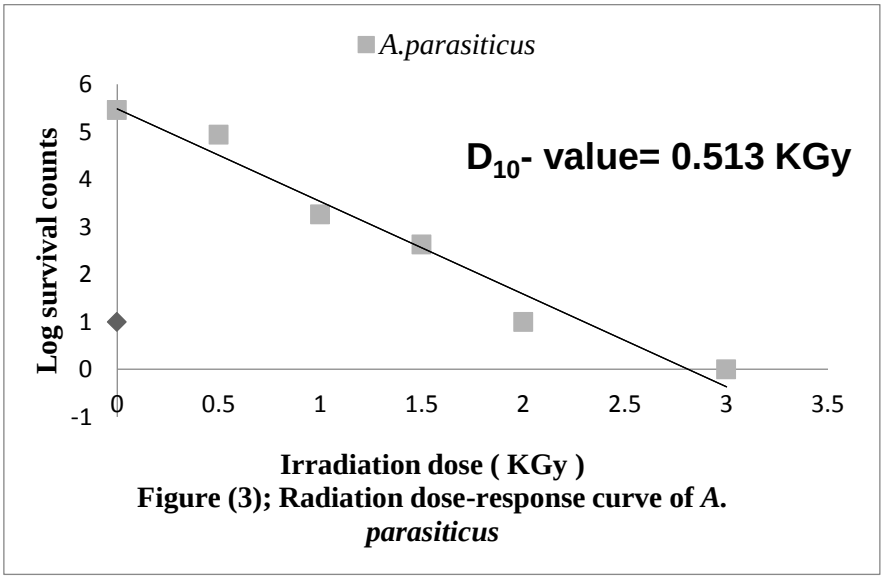

Figure (3); Radiation dose-response curve of *A. parasiticus*

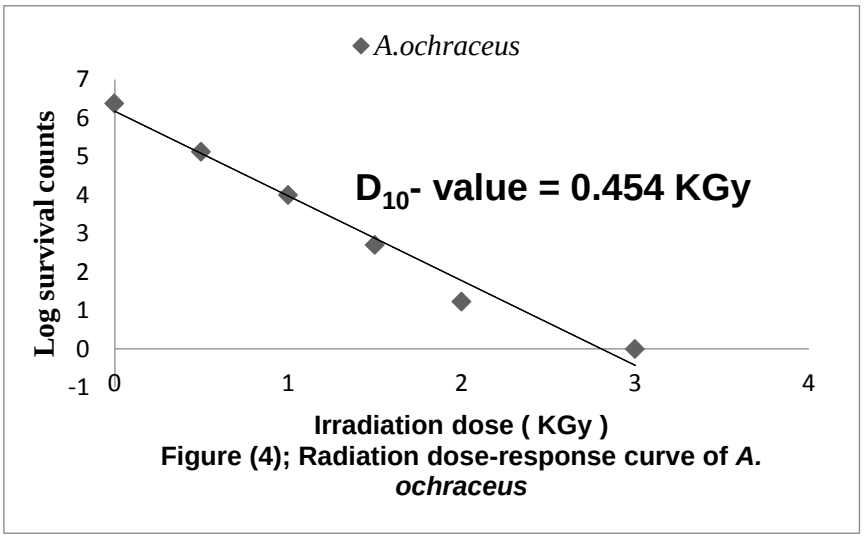

Figure (4); Radiation dose-response curve of *A. ochraceus*

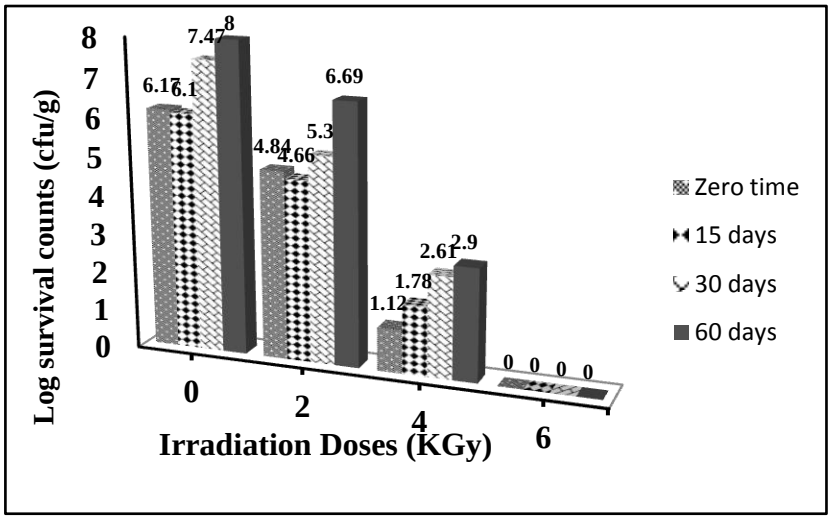

Fig. 5.

Effect of gamma irradiation on the *A. flavus and A.parasiticus* Logcounts (cfu/g) during storage periods (Zero time, 15 days, 30 days and 60 days).

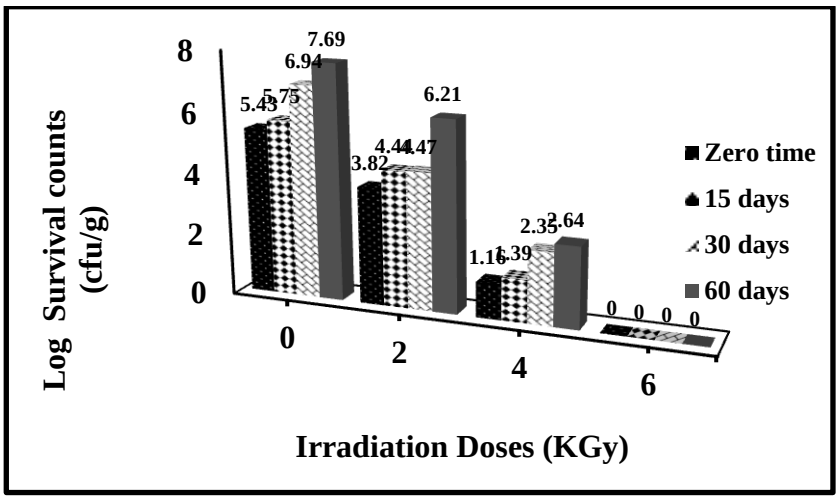

Fig. 6.

Effect of gamma irradiation on the *A. ochraceus* Logcounts (cfu/g) during storage periods (Zero time, 15 days, 30 days and 60 days).

Conclusion

Mold contamination of rice grains is a critical factor of great importance of assessing rice safety for human consumption. Irradiation doses 2.0 and 4.0KGy greatly reduced mycotoxin-producers mold counts, while 6.0 KGy completely prevented the growth of those molds. Irradiation doses of 4.0 and 6.0 KGy prevented the formation of aflatoxins while irradiation dose of 6.0 KGy prevented the production of OTA. Food irradiation technology successfully reduced mycotoxins formation, hence mitigate the risk problem resulting from mycotoxins contamination in rice and liquorice. In general application of irradiation technology provides safe and wholesome food products hence, protecting human and animal health.

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"تثبيط نمو الفطريات المُنتجة للسموم الفطرية في الأرز والعرق السوس عن طريق التشعيع لإختزال إنتاج السموم الفطرية

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الملخص العربي

عرفت العديد من الدراسات تلوث السموم الفطرية للأرز وعرق السوس كتهديد كبير لصحة الإنسان في الدول النامية. يمكن أن تصاب الحبوب والأعشاب الطبية بشدة بالعديد من الفطريات بسبب تنوع متغيرات الأرصاد الجوية في هذه المنطقة بالإضافة إلى ظروف التخزين غير الملائمة ، بما في ذلك درجات الحرارة المرتفعة والرطوبة ، والملوثات الطبيعية للمنتجات الزراعية المختلفة مثل *Aspergillus flavus, Aspergillus parasiticus, and Aspergillus ochraceus*. ، بالإضافة إلى السموم الفطرية الخاصة بهم ، التي تسبب مشاكل صحية للإنسان والحيوان. الهدف من هذه الدراسة هو دراسة تأثير جرعات أشعة جاما على نمو *A. flavus* و *A. parasiticus* و *A. ochraceus*التي تم تلقيحها صناعيا في الأرز وعرق السوس ، وكذلك على إنتاج السموم الفطرية والأوكراتوكسين. وجد أن جرعات التشعيع البالغة 2.0 و 4.0 كيلو جراي قللت بشكل كبير من الفطريات التي تؤدي لإنتاج السموم الفطرية و 6.0 كيلوجراي منعت نمو الفطريات تمامًا. جرعة التشعيع 4.0 كيلو جراي منعت تكوين الأفلاتوكسين ، بينما 6.0 كيلو جراي منعت إنتاج الأوكراتوكسين.و عليه ينصح بتشعيع الأرز و عرق السوس قبل تخزينة كما يجب المزيد من الدراسات مثل ذلك على حيوانات التجارب لدراسة أثر التشعيع على صحة و حياة حيوانات التجارب و من بعدها الإنسان.

الكلمات المفتاحية :*Aspergillus*, الحبوب , أشعة جاما , الاعشاب الطبية , الأختزال , السموم الفطرية.