Annals Of Agricultural Science, Moshtohor

Faculty Of Agriculture, Moshtohor . Benha University

ISSN:1110-0419

Faculty Of Agric. Moshtohor

VOL. 47 Number 4 December 2009

ISSN 1110-0419

Annals Of Agric. Sci., Moshtohor,
Vol. 47(4): Fo. 247-252, (2009).

USING GAMMA RADIATION AS ANTIFUNGAL AND ANTI-AFLATOXIGENIC AGAINST *ASPERGILLUS FLAVUS* ISOLATED FROM STORED CORN

BY

Gibriel, Y.A.Y.[1]*; Mohsen, S. M. and Akiela S. Hamza*****

* Department of Environmental Science, Faculty of Meteorology, Environment and Arid land Agriculture, King Abdulaziz University, Saudi Arabia,

** Department of Food Science & Technology, Faculty of Agriculture, Cairo University, Egypt, [3]Regional Center for Food and Feed, Agriculture Research Center, Egypt.

ABSTRACT

The effect of different gamma radiation doses on *Aspergillus flavus* growth and aflatoxin production in inoculated stored corn was applied and evaluated. *Aspergillus flavus* was isolated from stored corn and identified as an aflatoxigenic strain. 250 ml conical flasks each containing 100 gram of corn sterilized by autoclave and artificially inoculated with *Aspergillus flavus* approximately containing 10^6 cfu/ml were exposed to ^{60}Co gamma radiation for the appropriate time to obtain the required irradiation doses 3, 5 and 7 kGy. Results obtained for the *Aspergillus flavus* count after gamma irradiation indicated that gamma radiation at the three doses previously mentioned has a fungicidal or antifungal effect against *A. flavus* and its aflatoxin production up to 21 days of storage period. Corn treated with gamma radiation at a minimum dose of 3 kGy could be used as antifungal agents to manage the growth of *Aspergillus flavus* and aflatoxin formation in stored corn especially in granaries. Gamma radiation is safe, acceptable to consumers and extending the storage period of corn.

Key words: *Aspergillus flavus*, aflatoxin, gamma radiation, antifungal, stored corn.

INTRODUCTION

The fungi that produce aflatoxins especially *Aspergillus flavus* can infect important food and feed especially corn before, during and after harvest. These fungi are normal soil-borne inhabitants in our environment, growing on both living and decaying plant matter.

Food and Agricultural organization (FAO) estimated that as much as 25% of the world's agricultural commodities are contaminated with mycotoxins, leading to significant economic losses (Kabak *et al* 2006).

Many of the African countries have tropical climate with high ambient temperature and relative humidity all over the year and this provides optimal condition for the growth of toxigenic moulds, especially *Aspergillus flavus*. This leads to high fungal contamination in crops especially corn, wheat and barley (Bankole and Adebanjo 2003).

High fungal contamination deteriorates the quality of stored grains (Mislivec, 1979). The quality of the seed thus has an inverse relation with the number of fungal spores or colony especially in cases when these fungi have potential to produce mycotoxin (Aziz *et al*., 1997). *Aspergillus* sp. is reported one of the most notorious fungi found in corn and other grain products (Saleh *et al*., 1988; Emam *et al*., 1995).

1. **Correspondence now**: Y.A.Y.Gibriel, Lecturer, Microbiology Lab., Department of Environmental Sciences, King of Abdulaziz University, Elgamah, Jeddah, Saudi Arabia (e-mail: yousef1000@yahoo.com).

Recent evidence indicates that aflatoxins are associated with high incidence of primary liver cancer in parts of Africa, Southeast Asia and China (Gong *et al*., 2004; Jiang *et al*., 2005; Turner *et al*., 2007). The earlier correlation (Urrego Novoa and Diaz, 2006) led to the designation of aflatoxin B1 as a group 1A carcinogen by the International Agency for Research on Cancer (IARC, 1993). In 1982 twenty and tewleve cases were reported to cause aflatoxicosis and death, respectively, in Kenya. Aflatoxin was detected at 3.2-12mg/kg in food samples. Symptoms include: liver cancer, abdominal discomfort (Ngindu, 1982). FAO and WHO have imposed regulatory guidelines of 20 ppb of total aflatoxins as the maximum allowable limit in food or feed substrate. In some European countries aflatoxin levels are regulated below 5 ppb (Jiujiang *et al*., 2002).

Considering gamma-irradiation as practical mean for food decontamination, Gidding (1984) mentioned that the main objectives of food irradiation are to reduce post-harvest losses and enhance the safety and quality of food i.e to improve microbial quality of food.

Mohsen (1988) reported that because of increasing concern for possible health hazards associated with chemical residues, irradiation has been proposed as a replacement for these chemicals. The bases for this proposal results on the assumption that irradiated foods involve no toxicological health hazard to the consumer and since markets are becoming increasingly sensitive to chemical additives. Gamma irradiation eliminates or at least reduces the number of microbes down to a certain acceptable level in food items (FAO/IAEA/WHO ICGFI 1992).

The importance of this study comes from the wide spread of *Aspergillus flavus* producing aflatoxin in grains especially corn. In this paper, we report that gamma irradiation could also be used at a safety level dose to prevent the presence of *Aspergillus flavus* and prevent or to reduce its capability of producing aflatoxin . This could be done by using gamma irradiation at a safety level dose.

This will eliminate the corn associated toxin without compromising either health safety or grain food wealth.

Therefore the aim of the present work was planned to study the control of *Aspergillus flavus* by: Gamma irradiation using different doses of gamma radiation (3, 5 and 7 kGy), study its effect on fungal growth and the quantity of aflatoxin production.

MATERIALS AND METHODS

Organisms and inoculum preparation:

Aspergillus flavus was isolated from stored corn by placing 100g of stored corn in 500 ml bottle then 200 ml of Peptone buffer was added and then vortexed. Inoculation (2ml) from the previous preparation was made in RB (Rose Bengal chloramphenicol agar). Plates were incubated for 7 days at 25°C after this period, the spores were recovered by washing the RB surface with Peptone buffer. The harvested *Aspergillus flavus* spores were enumerated using a Brain Heart Infusion Broth and diluted to contain 10^6 cfu/ml approximately and the spore suspension was prepared for inoculation.

Controlling *Aspergillus flavus* by different doses of Gamma radiation:

The experiment was carried out using four 250 ml conical flasks each containing 100 gram of corn (Yellow corn variety Zea mays, obtained from Alexandria granaries, Egypt.) sterilized by autoclave at 121°C, 15 lbs/in^2 for 15 minutes. Moisture content was modified in the corn of each flask to reach 18%. This was done by adding the necessary calculated volume of sterilized distilled water. Each flask was inoculated by one ml *Aspergillus flavus* culture approximately (containing 10^6 cfu/ml). The first flask was kept as control (*Aspergillus flavus*) without exposed to gamma radiation. The other three flasks each was irradiated in a gamma chamber which has ^{60}Co gamma

source giving a dose rate of about 1.3636 kGy/h at the time of radiation. The required irradiation doses were 3, 5 and 7 kGy. Three replicates were prepared in each case. After irradiation all flasks were stored at 25°C for 21 days. Samples were taken before 0 day radiation, after 0 day and at 7, 14 days to determine *Aspergillus flavus* count and at 21 days to determine *Aspergillus flavus* count and aflatoxin production.

***Aspergillus flavus* count and identification:**

Five grams of each sample was added to a 45 ml of sterile diluting solution in 500 ml sterile Erlenmeyer flasks and homogenized thoroughly by vortex. Tenfold serial dilutions were then prepared. One ml portion of four suitable dilutions of the resulting sample suspension were used to inoculate petri dishes each containing 15 ml Rose Bengal chloramphenicol agar containing 0.5 mg Chloramphenicol per 500 ml solution to inhibit bacterial growth. Plates were then incubated for 7 days at 25 °C and the fungal growth was counted and identified in the same plates by the method of (Raper and Fennell 1965).

Aflatoxin production:

Extraction and determination of aflatoxins by thin layer chromatographic technique were conducted according to A.O.A.C. method (1990).

RESULTS AND DISCUSSION

The results indicated that *Aspergillus flavus* grew well in the control sample (*Aspergillus flavus* without being exposed to gamma radiation), increased from 4.78 Log cfu/g at 0 day before and after radiation to 5.38, 5.45 and 5.7 Log cfu/g at 7, 14 and 21 days storage period respectively, this caused an increase in aflatoxin production reached 220 ppb at 21 days storage period. On the other hand treatment with 3 kGy radiation dose destroyed *Aspergillus flavus* from 4.78 Log cfu/g at 0 day before gamma radiation) to undetected not only after the immediate subjection (0 day after gamma radiation) but also at all periods of storage 7, 14 and 21 days and prevented completely the aflatoxin production. Similar trend occurred when the radiation dose was raised up to 5 and 7 kGy. There was absolutely difference in *Aspergillus flavus* count and afltoxin production between the control sample and all treated samples with gamma radiation at all doses. (Fig 1& Table 1).

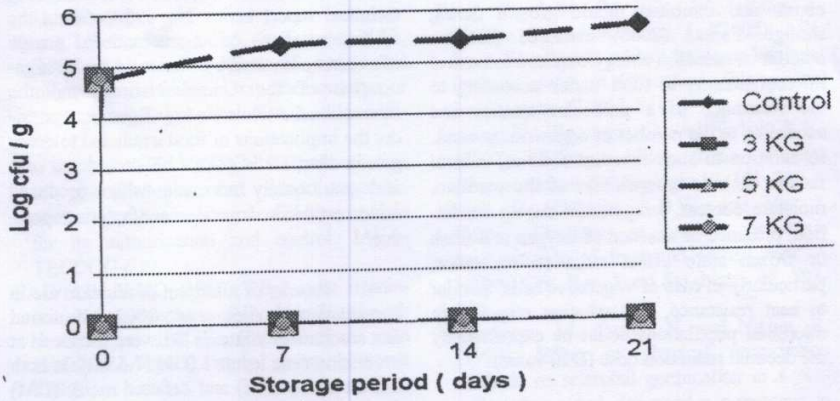

Fig. (1): Mean effect of different doses of gamma radiation on Aspergillus flavus count during storage at 25 °C of inoculated corn.

Table (1): Mean effect of different doses of gamma radiation on aflatoxin production during storage at 25°C of inoculated corn:

Gamma dose (KGy)	Days	Aflatoxin production (ppb)
0 (Control)	0	ND
	21	220
3	0 before treatment	ND
	21after treatment	
5	0 before treatment	ND
	21after treatment	
7	0 before treatment	ND
	21after treatment	

ND: not detected, ppb: part per billion

Gamma radiation was efficient to stop the growth of *Aspergillus flavus* in stored corn and aflatoxin production at a minimum dose of 3 kGy.

These results are in agreement with Menasherov *et al.* (1992) who reported that Sclerotia of *Aspergillus flavus* and *A. ochraceus* isolated from different cereals were not germinated following irradiation with 2.5 kGy. Results are also in agreement with Abd El –Aal and Aziz (1997) who found that doses of gamma-radiation of 1.0 - 3.0 kGy reduced the fungal growth and mycotoxins concentrations in grains. Gunes (2005) reported that irradiation of a variety of fruits at doses up to 3.5 kGy resulted in 3 log reduction in mould count, and inhibited mould growth during storage. Farkas (2006) indicated that the amount of radiation energy required to control microorganisms in food varies according to the resistance of a particular species and according to the number of organisms present. In addition to such inherent abilities, several factors such as composition of the medium, moisture content, temperature during irradiation, presence or absence of oxygen and fresh or frozen state affect radiation resistance, particularly in case of vegetative cells. Similar to heat resistance, the radiation response in microbial populations could be expressed by the decimal reduction dose (D10 value).

Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market (Pal & Gardener, 2006). Although restrictions are being imposed to protect food quality and the environment, chemicals are still our only recourse at present to prevent diseases of food crops. In recent years, the need to develop disease control measures as alternative to chemicals has become a priority of scientists worldwide (Raghavender, Reddy, & Reddy, in press). Radiation has been used since decades as an excellent tool for application in food sterilization, food preservation and different food engineering processes. This ultimately benefit the human society (Hyun-Pa *et al.*, 2006; Sameh *et al.*, 2006; Dus˘an, 2004; Ivanov *et al.*, 2001).

Furthermore FAO/IAEA/WHO (1999) technical report series No 890 presents the recommendations of an international groups of experts convened by tile world health organization of the United Nations and the International Atomic Energy Agency to consider the implications of food irradiated to doses greater than 10 kGy can be considered safe and nutritionally adequate when produced under established good manufacturing practice.

Results of aflatoxin production are in agreement with Aziz *et al.*, 2002, who found that amounts of aflatoxin B1 were enhanced at irradiation dose levels 1.0 and 1.5 kGy in both full-fat maize (FM) and defatted maize (DM) media and no aflatoxin B1 production at 3.0 kGy gamma-irradiation over 45 days of storage was observed.

Accordingly 3 KGy dose could be recommended for corn storage (granaries) as a mean of safety against the development of *Aspergillus flavus* growth and aflatoxin production.

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الأفلاتوكسين فى فطر *Aspergillus flavus* المعزول من الذرة المخزنة.

يوسف أحمد يوسف جبريل* صبحى محسن ** عقيلة صالح حمزة ***

* قسم العلوم البيئية، كلية الأرصاد والبيئة وزراعة المناطق الجافة، جامعة الملك عبد العزيز، المملكة العربيـــة السعودية.

** قسم الصناعات الغذائية،كلية الزراعة، جامعة القاهرة، مصر.

*** المركز اللاقليمى للأغذية والأعلاف، مركز البحوث الزراعية، مصر.

تم دراسة وتقييم تأثير الجرعات المختلفة لاشعة جاما على نمو فطر *Aspergillus flavus* وإنتاج الأفلاتوكسين فى الذرة الملقحة بة والمخزنة. حيث عزل الفطر من الذرة المخزنة وتم التعرف علية كسلالة منتجة للأفلاتوكسين . ولإجراء ذلك تم تلقيح ١٠٠ جرام من الذرة المعقمة والموضوعة فى دوارق مخروطية معقمة حجم ٢٥٠ مل وذلك بمعلق فطر *Aspergillus flavus* بنمو قدرة ١٠ ٦ مستعمرة/مل ثم التعريض لأشعة جاما من مصدر كوبالت ٦٠ وذلك للوقت المناسب بغرض الحصول على جرعات ٣و٥و٧ كيلو جراى. والنتائج المتحصل عليها لاعداد *Aspergillus flavus* بعد التعرض لأشعة جاما تدل على أن الجرعات الثلاث المستخدمة كا لها تأثير ضد انتاج الأفلاتوكسين *واستمر* التأثير حتى ٢١ يوم من التخزين. وتبين أيضا أن معاملة الذرة بالجرعة الدنيا وقدرها ٣ كيلو جراى يصلح كعامل مضاد لنمو فطر *Aspergillus flavus* ومنع تكوين الأفلاتوكسين *فى أماكن تخزين* الذرة. وتعتبر أشعة جاما عند الجرعة السابق ذكرها أمنة ويمكن استخدامها فى زيادة الفترة التخزينية للذرة.

حوليات العلوم الزراعية مشتهر

كلية الزراعة بمشتهر جامعة بنهـا

ISSN:1110-0419

المجلد السابع والأربعون - العدد الرابع - ديسمبر ٢٠٠٩ م