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Effect of gamma irradiation on the incidence and fumonisins production by *Fusarium* species occurring on maize and sorghum grains

Sreenivasa, M.Y.[1], Maheshwar, P.K.[3], Sanjay, K.R.[2], Diwakar, B.T.[2], Naidu, K.A.[2] and Janardhana, G.R.[3*]

[1]Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore, Karnataka State, India.
[2]Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore, Karnataka State, India.
[3]Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, Karnataka State, India.

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Maize and sorghum grain samples (250g) exposed to 2.5, 5.0, 7.5 and 10.0 kGy of gamma irradiation and samples were evaluated for the per cent incidence of *Fusarium* species and level of fumonisins at regular intervals of 0, 30, 60, and 90 days of storage. The results revealed that, on 0 day the incidence of *Fusarium* species was 48 and 38% in respective maize and sorghum samples and there was gradual decrease in the incidence of *Fusarium* species at 2.5 and 5.0 kGy doses of gamma irradiation after 30, 60, and 90 days storage. The incidence of *Fusarium* species was zero in samples treated at 7.5 kGy and 10.0 kGy doses of gamma irradiation. In contrast, there was gradual increase in the incidence of *Fusarium* species in unirradiated maize and sorghum samples. Further, analysis of maize and sorghum samples by HPLC revealed that, on 0 day the fumonisin concentration was 0.95µg/g and 0.49µg/g respectively. After 90 days of storage, the concentration of fumonisin was 0.98µg/g and 0.56µg/g in respective unirradiated maize and sorghum samples. The fumonisin content in irradiated samples was 0.94µg/g in 2.5 and 5.0 kGy, 0.93µg/g in 7.5kGy and 0.89µg/g 10.0 kGy and 0.56µg/g in 2.5 kGy, 0.54µg/g in 5.0 kGy, 0.53µg/g in 7.5 kGy and 0.52µg/g 10.0 kGy respectively. The study revealed that, 7.5 and 10 kGy gamma irradiation treatments were successful in preventing the growth of *Fusarium* species on maize and sorghum samples however, irradiation did not appear to be an effective method for eliminating fumonisins.

Key words: *Fusarium*, fumonisin, gamma irradiation, maize, sorghum

[*]Corresponding author: G.R. Janardhana; e-mail: grjbelur@gmail.com

Introduction

Maize (*Zea mays* L.) and Sorghum (*Sorghum bicolor* L.) are the two important cereal crops cultivated as major rain fed crops in most of the semi-arid regions. They are used in more ways than any other cereals by humans, as a feed grain, as a fodder crop, and for hundreds of industrial purposes (Hulluka and Esele, 1992; Anderson *et al.,* 2004). From the early stages of their growth and development, they are subjected to damage by many species of moulds including the species of *Fusarium* (Williams and McDonald, 1983; Bhattacharya and Raha, 2002). Therefore elimination of the growth of toxigenic *Fusarium* species is very important to prevent the subsequent production of fumonisins and dry matter loss in cereals used for human consumption (Miller, 2001).

An increased interest in the bio-preservation systems is responsible for the development of new variety of storage methods to prevent food spoilage by moulds. The major food preservation techniques such as low temperature, low water activity, acidification, etc. act by inactivating the spoilers, while most of the newer or emerging techniques of today like irradiation, electroporation, high hydrostatic pressure, etc. are act by directly inactivating microorganisms (Lavermicocca *et al.,* 2003). Consequently, there is an increase in the use of physical methods such irradiation to prevent food spoilage and storage losses because of their non-residual problems, uniform disinfection, economic feasibility and non toxic nature (Farkas, 1998). The ability of radiation to kill microorganisms has been investigated since the late 19^{th} century (Abd El-Aal and Aziz, 1997). In September 1997, study group appointed by WHO suggested that the food irradiation with doses less than 10 kGy can be considered as safe and nutritionally adequate when produced under established good manufacturing practices (Srinivas *et al.,* 1996; Rizk and Botros, 2006).

Gamma irradiation have been used to improve the quality and to extend the shelf life by destroying spoilage organisms, harmful insect pests and now it has become a potential food preservation technique. However, there are limited studies to know the effects of gamma irradiation to reduce the growth of *Fusarium* species and fumonisins production. Therefore, in the present investigation, naturally contaminated maize and sorghum grain samples exposed to different doses of gamma irradiation and evaluated for the incidence of *Fusarium* species and fumonisin levels.

Materials and methods

Sample collection

Freshly harvested maize and sorghum grains collected from farmer's fields (after drying) during the harvest season August – September 2006

were brought to the laboratory in sterile plastic bags and were used for irradiation studies.

Sample preparation for irradiation

Maize and sorghum samples (250 g) in triplicates were taken in 0.1mm thickness polythene bags (15 X 22cm) and sealed. The bags were subjected to gamma irradiation by exposing them to different doses of (2.5, 5, 7.5 and 10 kGray) of gamma irradiation at the rate of 200R/min. Samples were irradiated under continuous gamma sterilization plant (Model CGS 300, Make Baba Atomic Research Center, Mumbai) with 180-kilocurie with cobalt-60 (Co^{60}) source at Radiation Sterilization Plant, KIDWAI Memorial Institute of Oncology, Bangalore, Karnataka. After each treatment samples were stored at ambient (25$\pm$2 °C) conditions for 90 days. Samples were evaluated at regular intervals of 30 days for the incidence of *Fusarium* species and fumonisins levels.

Mycological analysis of samples

The percent incidence of *Fusarium* species on irradiated and unirradiated maize and sorghum samples were determined by agar plating method on Malachite green agar medium 2.5 (MGA 2.5) (Bragulat *et al.*, 2004). Briefly, 200 grains from respective samples were treated with 2% sodium-hypochlorite solution for 3 min., rinsed with sterile distilled water (three times) and plated on MGA 2.5 agar plates at the rate of 10 grains per plate. The plates were incubated under alternating periods of 12hrs darkness and 12 hrs of light at 25$\pm$2 °C for 7days. The samples were screened for the incidence of *Fusarium* species by microscopic observation. The per cent incidence of each *Fusarium* species was calculated (Ghiasian *et al.*, 2004).

Analysis of fumonisins by HPLC

The irradiated and unirradiated maize and sorghum samples (100 g) were ground to a fine powder in a fire proof mixer grinder (Kenstar Classique, MG-9605A), packed in plastic bags, labeled and stored at -20^0C until further analysis. The level of fumonisins was analyzed by HPLC with fluorescence detection system (Rice *et al.,* 1995). Finely ground sample (10 g) was transferred into a 250 ml conical flask and mixed with 50ml of acetonitrile/water (50/50, v/v). The conical flask was stoppered and shaken for 30 min. The mixture was filtered through Whatman No. 4 filter paper. The Sep-Pak C_{18} Cartridges (Waters Corporation, Massachusetts, Ireland) preconditioned with 2 ml ACN followed by 1% KCl solution. Filtrate (2 ml) was added to 6ml of 1% KCl and loaded into a C_{18} clean-up column. The solution was then passed through the Sep-Pak C_{18} Cartridge at a flow rate of 1 ml/min. The column was rinsed with 2 ml of 1% KCl followed by 2 ml of

ACN/ H_2O (15+85, v/v). The rinses were discarded, and air was forced through the column to expel all the rinse solution. FB_1 and FB_2 were finally eluted from the column with 2 ml of ACN/ H_2O (70+30, v/v). The elutants were evaporated to complete dryness under a gentle stream of nitrogen and dissolved in 100 µl of ACN/ H_2O (50+50, v/v) and subjected to HPLC analysis.

A sample (30 µl) eluted from the Sep-Pak C_{18} Cartridge was transferred to 1 ml microfuge tube to which, 30 µl of borate buffer, 30 µl of *ortho*-Phthalaldehyde (OPA) and 30 µl of water were added. The microfuge tube was capped, shaken briefly and allowed to react for 10min. at room temperature. A sample mixture (20µl) was injected into LC column (Schimadzu, Model SCL-10AVP) equipped with a loop size of 20µl, 150 X 4.6mm Phenomenex C_{18} analytical cartridge. The excitation was set at 335nm, emission at 440nm and flow rate was set at 1ml/min. The standards of fumonisin B_1 and B_2 (Sigma Aldrich Chemicals Pvt. Ltd.) were also injected to the HPLC prior to sample injection for programming. FB_1 and FB_2 were separated as sharp peaks with retention time of 4.62 and 9.97min respectively. The concentration of fumonisin was calculated by comparing peak areas samples with that of standards.

Results

The effect of different doses of gamma irradiation on per cent incidence of *Fusarium* species on maize and sorghum grains is presented in Figs. 1 and 2 respectively. On 0 day the incidence of *Fusarium* species was 48 and 38% in respective maize and sorghum samples. However, after 30 days of storage, the incidence of *Fusarium* species was reduced to 10% in 2.5 kGy, 1% in 5.0 kGy, 0% incidence in 7.5 kGy and 10.0 kGy irradiated maize samples. On 60 days of storage, the incidence of *Fusarium* species was 12% in 2.5 kGy, 1% in 5.0 kGy, 0% incidence in 7.5 kGy and 10.0 kGy irradiated maize samples. Similarly, after 90 days of storage, the incidence of *Fusarium* species was 17% in 2.5 kGy, 5% in 5.0 kGy, 0% incidence in 7.5 kGy and 10.0 kGy irradiated maize samples. In contrast, there was gradual increase in the per cent incidence of *Fusarium* species after 30, 60, and 90 days of storage in unirradiated maize samples. Eight different *Fusarium* species such as *Fusarium verticillioides, F. proliferatum, F. oxysporum, F. anthophilum, F. pallidoroseum, F. sporotrichioides, F. solani, F. decemcellulare* were recorded, of which *F. verticillioides* and *F. proliferatum* were the most dominant species (Table 1). Similarly, the incidence of *Fusarium* species on sorghum was 6% on 30 days in 2.5 kGy, 1% in 5.0 kGy and 0% incidence in 7.5 kGy and 10.0 kGy irradiated samples. On 60 days of storage, the incidence of *Fusarium* species reduced to 8% in 2.5kGy, 2% in 5.0kGy, 0% incidence in 7.5 kGy and 10.0 kGy irradiated sorghum samples respectively. On 90 days of storage, the incidence was 10% in 2.5 kGy, 2% in 5.0 kGy, 0%

in 7.5 kGy and 10.0 kGy on respective irradiated sorghum samples. In contrast, there was gradual increase in the incidence of *Fusarium* species after 30, 60, and 90 days of storage in unirradiated sorghum samples. *Fusarium* species such as *Fusarium verticillioides, F. proliferatum, F. oxysporum, F. anthophilum, F. pallidoroseum, F. sporotrichioides* and *F. solani* were recorded, *F. verticillioides* and *F. proliferatum* are the most dominant species (Table 1).

The effect of gamma irradiation on levels of fumonisin in maize and sorghum samples is presented in Table 2. Fumonisin level in un-irradiated maize and sorghum samples were 0.95 µg/g and 0.49 µg/g respectively. However, after 90 days of storage, the levels of fumonisin increased to 0.98µg/g and 0.56 µg/g in respect to maize and sorghum samples. In irradiated samples the fumonisin content was 0.94 µg/g in 2.5 and 5.0 kGy irradiation, 0.93µg/g in 7.5 kGy irradiation and 0.89 µg/g in 10.0 kGy irradiation after 90 days of storage. However Fumonisin level was reduced to 0.56 µg/g in 2.5 kGy, 0.54 µg/g in 5.0 kGy, 0.53µg/g in 7.5 kGy and 0.52 µg/g 10.0 kGy in irradiated sorghum samples respectvily. The study showed that, there was no significant reduction in fumonisin levels in irradiated and unirradiated maize and sorghum samples when the doses of gamma irradiation increased.

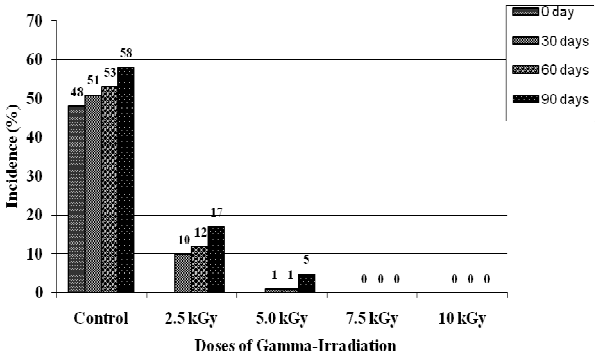

Fig. 1. Effects of gamma irradiation on percent incidence of *Fusarium* species on maize samples.

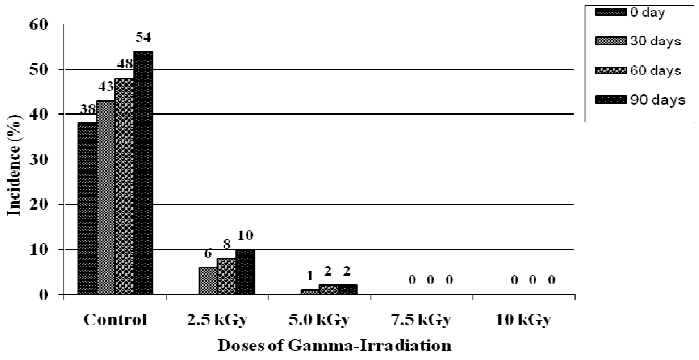

Fig. 2. Effects of gamma irradiation on per cent incidence of *Fusarium* species on sorghum samples.

Discussion

The effect of gamma-irradiation in the decontamination of food commodities has been reported by several investigators all over the world (Lagunas-solar, 1995; Farkas, 1998; Aziz and Moussa, 2002; Prado *et al.,* 2003). The gamma-irradiation dose of 7.5 to 10 kGy is sufficient to sterilize food and feed products (Andrews *et al.,* 1998). Aziz *et al.* (1999) found that gamma-irradiation is known to delay the mycelial growth of fungi and mycotoxin production and also reported that, the total viable population of fungi and toxin production decreased significantly with increasing gamma-irradiation doses.

This study was conducted to know the effect of gamma irradiation on the per cent incidence of *Fusarium* species on maize and sorghum showed promising results. The data showed that, the incidence of *Fusarium* species decreased significantly with increasing doses of gamma irradiation from 5 to 10 kGy. Many of the *Fusarium* species completely killed or eliminated at radiation dose of 7.5 and 10 kGy respectively. The sensitivity of fungi to gamma-irradiation has been established by Saleh and Aziz (1996) and Abd El-Ad and Aziz (1997) who recorded that the minimum dosage required for complete inhibition of fungi in different food and feed products ranged from 4 to 6 kGy. There are number of reports which suggested that moulds are very sensitive to gamma-radiation (Refai *et al.,* 1996; Youssef *et al.,* 1999). El-Samahy *et al.* (1995) reported that the total moulds on maize grains decreased with increased radiation doses. In the present study, *F. verticillioides F. oxysporum* and *F. anthophilum* showed resistance up to 5 kGy dose of gamma irradiation, while the *F. proliferatum, F. pallidoroseum* and *F. solani* showed resistance up to 2.5 kGy dose of gamma irradiation. O'Neill *et al.* (1991) found that *Fusarium* and *Alternaria* species are more resistant to irradiation than *Aspergillus* and *Penicillium* species. Irradiation of grains at doses of 2.0 and 4.0 kGy significantly decreased the total fungal counts

compared to unirradiated controls. Rizk and Botros (2006) also reported that *Aspergillus candidus, A. granulosis* and *Curvularia geniculata* are the most sensitive species in gamma irradiation of 1.5 kGy, while the most resistant species were *Fusarium moniliforme, F. oxysporum* and *F. solani* showed resistance up to 6.5 kGy irradiation.

Effect of gamma irradiation on the extent of fumonisins levels revealed that, there was no significant reduction in the levels of fumonisins in irradiated and unirradiated maize and sorghum samples. D'Ovidio (2005) reported that irradiation has no effect on FB_1 in both the ground and whole kernel corn. There are number of reports on zero effect of irradiation on mycotoxins and conflicting reports on the influence of irradiation on detoxification of mycotoxins (Hooshand and Klopfenstein, 1995). Paster *et al.* (1985) indicated that pure ochratoxin is stable even at 75 kGy of irradiation. Kume *et al.* (1987) found that the dosage required for pure aflatoxin destruction was greater than 50 kGy. Refai *et al.* (1996) showed that a dose of 15 or 20 kGy was sufficient for complete destruction of ochratoxin A in yellow corn and soybean. Aziz and Youssef (2002) showed that application of radiation at 10 kGy significantly detoxified aflatoxin B_1 by 82-88% and zearalenone by 88-94% but only by 44-48% ochratoxin and a dose of 20 kGy was sufficient for complete destruction of aflatoxin B_1 and zearalenone and reduced the amount of ochratoxin A in foods by 72-76%. Recently, Aquino *et al.* (2005) reported that gamma irradiation with C^{60} was found to be effective in reducing the incidence of *A. flavus* in the maize samples. Furthermore, he has also reported that the irradiation induced a partial reduction in aflatoxin B_1 and B_2 levels with irradiation doses of 2, 5 and 10 kGy. In this study, high dosage of gamma irradiation not able to eliminate fumonisin content completely in maize and sorghum grains irradiated with 2.5 to 10 kGy of gamma irradiation. These results are in accordance with those obtained by Aziz *et al*. (2006), D'Ovidio (2005) Etcheverry *et al.* (1998), Farias *et al.* (2000) and Owolade *et al.* (2000). The results of the present investigation suggest that gamma irradiation could be used to reduce the percent incidence of *Fusarium* species causing deterioration of cereals. This study also indicated that irradiation is an effective method to remove moulds from naturally contaminated maize and sorghum samples. However, irradiation not appears to be an effective method for eliminating fumonisins in contaminated maize and sorghum grains intended for human and animal consumption.

Table 2. Effect of gamma irradiation on levels of fumonisins in maize and sorghum grains.

S1. No.	Doses of gamma-irradiation	0 day			90 days		
		FB_1	FB_2	Total	FB_1	FB_2	Tatal
		(µg/g)					
		Maize					
1	control	0.95	0.00	0.95	0.98	0.01	0.99
2	2.5 kGy	0.95	0.00	0.95	0.94	0.00	0.94
3	5.0 kGy	0.95	0.00	0.95	0.94	0.00	0.94
4	7.5 kGy	0.95	0.00	0.95	0.93	0.00	0.93
5	10.0 kGy	0.95	0.00	0.95	0.89	0.00	0.89
		Sorghum					
1	control	0.484	0.006	0.49	0.56	0.00	0.56
2	2.5 kGy	0.484	0.006	0.49	0.56	0.00	0.56
3	5.0 kGy	0.484	0.006	0.49	0.54	0.00	0.54
4	7.5 kGy	0.484	0.006	0.49	0.53	0.00	0.53
5	10.0 kGy	0.484	0.006	0.49	0.52	0.00	0.52

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Table 1. Effects of gamma-irradiation on incidence (%) of different species of *Fusarium* on maize and sorghum grains.

G I (Doses)	*Fusarium* species																											
	F. verticillioides				*F. proliferatum*				*F. oxysporum*				*F. anthophilum*				*F. pallidoroseum*				*F. sporotrichioides*				*F. solani*			
	(Storage days)																											
	0	30	60	90	0	30	60	90	0	30	60	90	0	30	60	90	0	30	60	90	0	30	60	90	0	30	60	90
Maize																												
Control	25*	26	30	28	11	13	15	12	01	11	08	09	06	02	05	07	03	-	01	-	-	-	-	01	01	09	04	01
2.5 kGy	25	04	06	09	11	02	02	04	01	03	01	-	06	03	02	04	03	01	-	-	-	-	-	-	01	-	01	-
5.0 kGy	25	-	01	03	11	-	-	-	01	-	-	01	06	01	-	-	03	-	-	01	-	-	-	-	01	-	-	-
7.5 kGy	25	-	-	-	11	-	-	-	01	-	-	-	06	-	-	-	03	-	-	-	-	-	-	-	01	-	-	-
10 kGy	25	-	-	-	11	-	-	-	01	-	-	-	06	-	-	-	03	-	-	-	-	-	-	-	01	-	-	-
Sorghum																												
Control	22*	21	27	28	07	09	02	14	02	05	04	03	03	05	12	02	01	02	02	-	02	01	01	01	01	-	-	06
2.5 kGy	22	05	05	07	07	-	01	02	02	-	-	-	03	01	01	01	01	-	-	-	02	-	01	-	01	-	-	-
5.0 kGy	22	-	01	01	07	-	-	01	02	01	-	-	03	-	01	-	01	-	-	-	02	-	-	-	01	-	-	-
7.5 kGy	22	-	-	-	07	-	-	-	02	-	-	-	03	-	-	-	01	-	-	-	02	-	-	-	01	-	-	-
10 kGy	22	-	-	-	07	-	-	-	02	-	-	-	03	-	-	-	01	-	-	-	02	-	-	-	01	-	-	-

Note: G I, Gamma irradiation; *Average of 2 single analyses of 2 treated samples; - No incidence.