Effect of γ -irradiation on fumonisin producing *Fusarium* associated with animal and poultry feed mixtures

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Received: 8 February 2017 / Accepted: 15 March 2017 / Published online: 25 April 2017
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Abstract Contamination of animal and poultry feeds by *Fusarium* and the mycotoxin Fumonisin B1 is frequent in the feed supply chain. The present study evaluated the prevalence of fumonisin B1 producing *Fusarium* among irradiated and non-irradiated animal and poultry feed mixtures. Further, the efficiency of γ -rays (2.5, 5.0, 7.5, and 10.0 kGy) to minimize *Fusarium* growth and biosynthesis of fumonisin B1 in artificially inoculated feed was evaluated. A total of 108 feed samples were collected in which 45.37% of feed mixtures were contaminated with *Fusarium* species. Among the contaminated samples, the frequency levels of *F. verticillioides* and *F. proliferatum* were 42.59 and 24.07%, respectively. Out of the 98 *Fusarium* isolates from feed samples, 84.7% of *F. verticillioides* and 64.28% of *F. proliferatum* were positive for FUM1 set of primers. Fumonisin B1 biosynthesis by the FUM1 positive isolates in feed was confirmed by LC/MS which recorded 0.1–45 $\mu\text{g/g}$ of feed. Fungal growth and viable count of *Fusarium* in PDA medium and feed decreased with increasing irradiation dosage. Interestingly, fumonisin content was 11 $\mu\text{g/g}$ of feed in 2.5 kGy irradiated sample as compared to 5 $\mu\text{g/g}$ of feed in non-irradiated control. Ionizing radiation at 7.5 kGy was found lethal for fungal growth and fumonisin production. Our findings suggest that γ -radiation above 7.5 kGy effectively prevented fungal growth in feed mixtures and minimized the

exposure of animal and human life to the potential risk of mycotoxin. Also it is necessary to maintain proper storage system for feeds until consumption.

Keywords *Fusarium* · Fumonisin B1 · Animal feed · Poultry feed · γ irradiation

Introduction

Animal and poultry feed industry has transformed into a well-organized, scientifically, oriented and technologically driven sector owing to its domestic and international market. India is the third largest egg producer and ranks fifth among meat producers globally. Feed being a critical component of food chain, its possible contamination by fungi and mycotoxins has a direct impact on animal health and also on food safety and public health. *Fusarium* species are the common contaminants of maize-based feed mixtures and it entails risk to human and animal health by producing fumonisins (Glenn 2007). *Fusarium verticillioides* and *F. proliferatum* are the most important producers of fumonisin toxins with wide geographical distribution and frequent occurrence in maize and maize-based food/feeds. Fumonisins are divided into four groups: A, B, C, and G, with the B-type fumonisins (FB1 and FB2) being the most toxic. FB1 makes up approximately 70% and the others 10–20% of the total fumonisin content (Nelson et al. 1993). FB1 toxin is known to cause animal mycotoxicoses such as equine leukoencephalomalacia in horses, porcine pulmonary edema in swines, and diarrhea, weight loss, inflamed liver, and poor performance in poultry (Marasas 1995; Prathap Kumar et al. 1997; Hollinger and Ekperigin 1999). The major problem associated with mycotoxin-contaminated feeds is not the acute disease episodes, but low level toxin ingestion that causes metabolic

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disturbances resulting in poor animal performance and productivity (Bryden 2012). The US Food and Drug Administration (FDA) has set a permissible limit of total fumonisins in maize and maize-based products at 2–4 µg/g for foods and 5–100 µg/g for animal feeds (FDA 2001). The European Union Commission has regulated fumonisin levels to 1 mg/Kg for maize-based foods and 4 mg/Kg for unprocessed maize (European Commission 2007). Food and Drug Administration has set up the guidance levels for total fumonisins (FB1, FB2, and FB3) as 100 ppm in grain and grain by-products and 50 ppm in complete diet for poultry (FDA 2011).

Despite the application of various physical and chemical treatments to limit the fungal growth and mycotoxin production, complete eradication of toxins is challenging as contamination by fungi and mycotoxins can occur during storage or at any time until consumption. Several substantial efforts have been undertaken to avoid contamination, but the most versatile is the treatment with ionizing radiation, particularly γ -irradiation in food and feed products (Ferreira-Castro et al. 2007). Low radiation dosage (up to 10 kGy) did not hamper nutritional quality of grains and feeds, but a few vitamins of the food grains are sensitive to irradiation. The nutritional loss (when treated with >10 kGy) is of minor importance when compared to the depletion in nutrients observed during heating of the food products (WHO 1999; Grolichova et al. 2004). An average dosage of 10 kGy irradiation is widely used and significantly reduces fungal population in maize and maize-based food products. Besides, a controlled storage environment is also of paramount importance for ensuring food safety. So far, only a few studies have reported fungal contamination and the presence of mycotoxins in irradiated maize. Moreover, to the best of our knowledge, no previous studies have analyzed fungal contamination of poultry feeds treated with higher doses of γ -irradiation. Here, we evaluated irradiated and non-irradiated animal and poultry feeds collected from various districts of Karnataka, India for contamination by *Fusarium*. PCR-based screening was performed for the identification of *Fusarium* species and FUM1 gene. Additionally, the ability of *Fusarium* isolates to produce FB1 was analyzed by artificial culturing in feed mixtures and detecting the FB1 levels by LC/MS. The study also evaluated the effect of different doses of γ -irradiation on *Fusarium* growth and FB1 production in feed model.

Materials and methods

Collection of feed mixtures

Animal feeds and poultry feed mixtures were collected from various markets, local stores, feed manufacturers,

poultry, and animal farms covering 15 districts (Bellary, Bengaluru, Chamarajanagar, Dawanagere, Dharwad, Haveri, Hassan, Kodagu, Koppal, Mandya, Mysuru, Raichur, Ramanagara, Shimoga, and Tumkur) of Karnataka, India. A total of 108 feed mixtures (<500 g) comprising 52 animal feeds and 56 poultry feeds were collected among which 9 animal feeds and 14 poultry feeds were irradiated. Feed mixture samples were brought directly to the laboratory in sterile polythene bags and maintained at 4 °C until analysis.

Mycological analysis of feed mixtures

Each feed sample was ground using sterile pestle and mortar and serially diluted followed by spread-plating on potato dextrose agar (PDA) medium and Malachite green oxalate Agar (MGA) 2.5 medium. The MGA medium is the selective medium for *Fusarium* species. The plates were incubated under 12-h light/dark conditions at 28 ± 2 °C for 7 days. Fungal colonies that appeared on the plates were extensively studied for micro-morphological characteristics of *Fusarium* species with special emphasis on *F. verticillioides* and *F. proliferatum* using fungal keys and manuals (Leslie and Summerell 2006). All *Fusarium* isolates were sub-cultured onto Czapek Dox Agar (CZA) slants, incubated at 28 ± 2 °C for 7 days and maintained at 4 °C for further studies. Percent frequency of feeds contaminated with *Fusarium* species was determined by applying the following formula: % frequency = [No. of feed samples contaminated with *Fusarium* species/Total no. of feed samples analyzed] $\times$ 100.

DNA extraction and PCR analysis of *Fusarium* species

Genomic DNA was extracted from all *Fusarium* isolates by the conventional phenol–chloroform method (Sreenivasa et al. 2008).

The species of *Fusarium* isolates were identified by PCR using primers targeting the species-specific region of IGS of *F. verticillioides* and *F. proliferatum*. These isolates were also subjected to PCR screening for the presence of FUM1 gene which encodes a polyketide synthase that catalyzes the synthesis of a linear polyketide which forms the backbone of fumonisins. Amplifications were carried out in 25 µL reaction mixture which contained 2.5 µL of 10X PCR buffer, 0.2 mM dNTPs, 2 mM MgCl₂, 0.6 µM each of forward and reverse primers and 0.625 units of Taq DNA polymerase (Genei, Bengaluru, India). VERTF1 (5'-GCG GGA ATT CAA AAG TGG CC-3') and VERTR (5'-CGA CTC ACG GCC AGG AAA CC-3') were used for the identification of *F. verticillioides* (Sreenivasa et al. 2008). PCR conditions involved initial denaturation at 95 °C for

2 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 61 °C for 30 s, extension at 72 °C for 1 min, and a final elongation at 72 °C for 13 min. Primer pairs, Fp3-F (5'-CGG CCA CCA GAG GAT GTG-3') and Fp4-R (5'-CAA CAC GAA TCG CTT CCT GAC-3'), described by Jurado et al. (2006) were employed for the identification of *F. proliferatum*. The thermal cycling conditions involved initial denaturation at 94 °C for 1 min 25 s followed by 35 cycles of denaturation at 95 °C for 35 s, annealing at 68 °C for 30 s, extension at 72 °C for 1 min, and a final elongation at 72 °C for 5 min. *F. verticillioides* MTCC 1848 and *F. proliferatum* MTCC 286 were used as reference strains.

Detection of FUM1 gene was performed using the primers rp32 (5'-ACA AGT GTC CTT GGG GTC CAG G-3') and rp33 (5'-GAT GCT CTT GGA AGT GGC CTA CG-3') (Proctor et al. 2004). PCR conditions were 94 °C for 30 s, 30 cycles of 94 °C for 30 s, 57 °C for 30 s, 72 °C for 1 min, and 72 °C for 5 min. The amplified products of *F. verticillioides* and *F. proliferatum* were sequenced (Amnion Biosciences, Bengaluru, India) and their identities were confirmed by BLAST analysis. The respective nucleotide sequences were submitted to NCBI GenBank. The phylogenetic tree was constructed to provide an overview of the genetic relatedness of IGS sequences of the representative *Fusarium* isolates using MEGA 5.1 software by neighbor joining method.

Detection of fumonisin by LC/MS

FB1 analytical standard was purchased from Cayman Chemical (Michigan, USA) and a standard solution was prepared by dissolving toxin standard in acetonitrile/water (1:1) to get a concentration of 1.0 mg/mL. Three FUM1 positive isolates of both *F. verticillioides* and *F. proliferatum* were randomly selected and tested for their ability to biosynthesize FB1. One mL (10^6 spores/mL) of each *Fusarium* isolate was artificially inoculated into 5.0 g autoclaved poultry feed mixture in culture tubes. Culture tube without fungal inoculation was used as control. The tubes were incubated in dark for one month. After incubation, feed mixtures were freeze-dried and finely ground in liquid nitrogen gas using a chilled, sterile pestle and mortar and stored at -80 °C until it is processed for FB1 extraction. The powdered sample (0.4 g) was taken in a sterile glass vial and suspended in 2.0 mL acetonitrile/water (1:1) and allowed for equilibration overnight in a gel rocker at 28 ± 2 °C. The extracts were syringe-filtered using 0.45 µm nylon membrane filters and subjected to liquid chromatography/mass spectrometry (LC/MS) (Waters Acquity/SYNAPT G2, USA). Chromatographic separation was achieved on a C18 column maintained at 50 °C. Mobile phase A was 0.3% formic acid in water (v/v)

and acetonitrile being mobile phase B. Twenty microliter sample was injected and processed for 8 min. The mass spectrometer was operated in the positive electrospray ionization mode (ESI+). The capillary voltage was set at 1.8 kV and the cone voltage was 40 V. The source temperature was 100 °C, and the desolvation temperature was maintained at 200 °C. The desolvation gas flow rate was 500 L/h, and Helium was used as collision gas. The limit of detection (LOD) for FB1 was 10 ppm. MassLynx SCN781 software was used to validate the LC/MS results.

Effect of γ -irradiation on growth and FB1 production by *F. proliferatum*

Γ -ray effect on *Fusarium* growth and FB1 production was studied in PDA medium and in poultry feeds, respectively, according to Mansur et al. (2014) with slight modification. PDA plates were inoculated with 7.5 mm diameter *F. proliferatum* disc. The plates were incubated at 28 ± 2 °C for 24 h and were irradiated at 2.5, 5.0, 7.5, and 10.0 kGy at a chamber temperature of 33.8 °C in a ^{60}Co γ irradiator (γ chamber 1200, BRIT, Mumbai) at a dose rate of 9.352 kGy/h. Non-irradiated sample was the control. After treatment, the plates were incubated at 28 ± 2 °C and evaluated for fungal growth at day 5 and 10. Similarly, culture tubes containing 5.0 g of poultry feed ($a_w = 1$, autoclaved at 121 °C for 20 min) were inoculated with 100 µL of *F. proliferatum* spore suspension (10^6 spores/mL) and incubated at 28 ± 2 °C for 24 h. The culture tubes were then irradiated as mentioned above. The non-irradiated tube was used as control. After irradiation, the samples were incubated at 28 ± 2 °C for 21 days and analyzed for fumonisin. The colony forming units (CFU) of *Fusarium* were determined by serially diluting the treated poultry feed and plating on PDA plates. Fumonisin was extracted from the irradiated poultry feeds and subjected to detection as described earlier.

Statistical analysis

Experiments were performed in triplicates and the results were expressed as mean $\pm$ standard deviation. The graphs were drawn using GraphPad prism version 5.03 software.

Results and discussion

Contamination of feed mixtures by *Fusarium* species

Cereal grains and their by-products are the major formulations involved in feed production units, among which maize is the chief ingredient. *Fusarium* species are the predominant fungal contaminant of maize and maize-based

Table 1 Contamination of animal and poultry feeds with *Fusarium* species

	Animal feeds		Poultry feeds	
	Irradiated	Non-irradiated	Irradiated	Non-irradiated
Total feeds collected	9	43	14	42
No. of feeds contaminated with <i>F. verticillioides</i>	4	8	9	25
No. of feeds contaminated with <i>F. proliferatum</i>	NC	5	3	18
No. of feeds without <i>Fusarium</i> contamination	5	30	2	16

NC no contamination

Fig. 1 PCR amplification of *Fusarium verticillioides* isolates (amplicon size of 1016 bp), *Fusarium proliferatum* isolates (amplicon size of 230 bp), and FUM1 gene (amplicon size of 680 bp). **a** Lane M is a 100 bp DNA ladder, Lane 1 positive control, *F. verticillioides* MTCC1848, Lane 2–16 *F. verticillioides* isolates, Lane 17 negative control, *Aspergillus flavus*; **b** Lane M & Lane 17 100 bp DNA ladder, Lane 1 positive control, *F. proliferatum* MTCC 286, Lane 2–15 *F. proliferatum* isolates, Lane 16 negative control, *Aspergillus flavus*; **c** Lane M is a 100 bp DNA ladder, Lanes 1, 2, 3, 6, 7, 8, 10, 12, & 13 positive isolates for FUM1 gene, Lanes 4, 5, 9, 11, & 14 negative isolates for FUM1 gene

food/feed products resulting in contamination by fumonisins which has adverse health and economic effects. Also, maize grains are rich in starch content and represent an excellent substrate for *Fusarium* growth and fumonisins synthesis. A study by Pereyra et al. (2011) reported high prevalence of *F. verticillioides* and *A. flavus* in the raw materials and finished feeds intended for fattening pigs, and HPLC detection revealed that all the samples were positive for fumonisin B1. Monge et al. (2012) reported the co-occurrence of *A. flavus* and *F. verticillioides* in the raw materials and poultry feeds of Cordoba, Argentina.

In our study, preliminary identification based on the micro-morphological features revealed that the fungi were *Fusarium*. Out of 108 feed mixtures collected, 49 feed samples were contaminated by *Fusarium* species showing a percent frequency of 45.37% in which 13 samples were animal feeds and 36 were poultry feeds. Among the 49 contaminated feed mixtures, 46 feeds were contaminated with *F. verticillioides* (42.59%), 26 feeds were infected

with *F. proliferatum* (24.07%), and other *Fusarium* species showed a frequency of 11.1% contaminating 12 feed mixtures. Among the 9 irradiated animal feeds, 4 samples showed incidence of *F. verticillioides*, whereas *F. proliferatum* was completely absent. In the irradiated poultry feeds, 9 and 3 samples showed contamination with *F. verticillioides* and *F. proliferatum*, respectively (Table 1).

A total of 98 *Fusarium* isolates obtained from the contaminated samples were subjected to species identification as well as for screening of FUM1 gene. PCRs using species-specific primers have confirmed that 72 isolates were *F. verticillioides* and 14 were *F. proliferatum* (Fig. 1). Of the 72 isolates of *F. verticillioides*, 61 amplified FUM1 gene and among the 14 isolates of *F. proliferatum*, 9 scored positive for the presence of FUM1 gene (Fig. 1). Representative amplicons were sequenced and confirmed their identity by BLAST search. Sequences of *F. verticillioides* have been submitted to GenBank under the accession numbers KJ159073, KR071794, KR071795, KR071796,

Fig. 2 Phylogenetic tree showing relationship between different isolates of *F. verticillioides* and *F. proliferatum*

KR071797, and KR071798 and that of *F. proliferatum* under the accession numbers KJ159072, KR071799, and KR071780. The relationship of the sequenced *Fusarium* isolates has been represented in the dendrogram based on the BLAST algorithm (Fig. 2). We have also identified other species of *Fusarium* such as *F. globosum* ($n = 4$), *F. oxysporum* ($n = 6$), and *F. anthophilum* ($n = 2$) on the basis of their phenotypic features.

The problem of fungal contamination of feeds is challenging in India owing to non-scientific methods of agricultural practices, improper storage system, and unfavorable environmental conditions. Few studies have reported the incidence of *Fusarium* in animal and poultry feeds from India. A high prevalence (84%) of *Fusarium* species was reported among the poultry feed samples from Haryana (Jindal et al. 1999). Study conducted by Dass et al. (2007) identified high incidence (89.09%) of *F. verticillioides* in animal and poultry feeds in Karnataka. A study by Deepa et al. (2016) showed that 69 cereal samples out of 135 collected from different districts of Karnataka were contaminated by *Fusarium* species with *F. verticillioides* being predominant. The present study also revealed that *F. verticillioides* is the most frequently occurring FB1 producing fungus in the feed mixtures collected from different districts of Karnataka. Pacin et al. (2003) showed that maize-based pelleted feed samples of coastal and mountain regions of Ecuador were contaminated by *Aspergillus flavus*, *A. ochraceus*, *Fusarium graminearum*, *F. verticillioides*, *F. semitectum*, and *F. oxysporum*. Labuda et al. (2003) reported an incidence rate of 49% of *Fusarium* contamination among the feeds analyzed from Slovakia and observed the predominance of *F. proliferatum* (584 isolates out of the 609 *Fusaria*). Contamination of the irradiated feed samples analyzed in our study raises concern and this could probably be due to poor quality of raw materials, improper storage, and handling in poultry farms and local markets. Additionally, detection of the stage of contamination and taking appropriate measures would reduce the toxigenic fungi in the feeds.

LC/MS analysis for FB1

To confirm the biosynthesis of FB1 by FUM1 positive isolates of *F. verticillioides* and *F. proliferatum*, 3 isolates of both the species were assessed by LC/MS for their ability to produce FB1 in feed. The standard FB1 toxin had a retention time of 1.7 min (Fig. 3). All the 3 isolates of *F. verticillioides* produced FB1 in feed to the extent of 45, 0.2, and 11 $\mu\text{g/g}$ of feed. The PCR results of the isolates correlated with the quantitative analysis of FB1. In *F. proliferatum*, one among the 3 isolates produced 0.1 $\mu\text{g/g}$ of FB1 in feed. However, FB1 was not detected in other two *F. proliferatum* isolates in spite of harboring FUM1 gene. This could be due to unfavorable environment or FUM1 gene of the isolates might have failed to express the gene in feed sample or FB1 produced by the isolate could be below the detection limit. Control without inoculation showed no FB1 content on LC/MS analysis thus confirming no pre-occurrence of fumonisins in feed (Fig. 3). Sanchez-Rangel et al. (2005) reported the association of FUM1 gene with fumonisin production in 54 isolates of *F. verticillioides*, except in seven that did not synthesize FB1 in spite of containing the gene. Another study reported that all the *F. proliferatum* (40.9%) isolates from asparagus contained FUM1 and FUM8 genes and also produced fumonisins ranging from 28 to 4202 $\mu\text{g/g}$ in maize cultures (Wang et al. 2010).

Effect of γ -irradiation

Ionizing radiation is widely used for the preservation of food and feed commodities and is considered as an important measure of decontamination. Radiation doses up to 10 kGy are effective in microbial decontamination and pose no toxicological hazard or nutritional and microbiological problems in foods (Aziz et al. 2006). In the present study, fungal growth in the PDA plates decreased with the increase in irradiation dosage when observed at two incubation intervals (Fig. 4). Mycelial growth was delayed in the 5 kGy treatment while complete absence of growth was

Fig. 3 LC/MS analysis of FB1 at a retention time of 1.76 min having a molar mass of 722 g/mol. **a** MS profile of FB1 standard; **b** chromatogram of FB1 standard; **c** MS profile of control; **d** MS profile of FB1 from feed sample

Fig. 4 Effect of gamma irradiation on the growth of *F. proliferatum*

recorded at 7.5 and 10 kGy dosages. Similar results were obtained in feed substrates, in which the viable counts of *F. proliferatum* decreased with increasing radiation dosage (Fig. 5).

The lethal dose was found to be 7.5 kGy. Water activity of the feed was maintained the same ($a_w = 1.0$) before and

after irradiation of the feed samples. The largest number of CFU/g (1.2×10^3) was observed in the non-irradiated control. Ferreira-Castro et al. (2007) studied the effect of γ -irradiation on maize artificially cultured with *F. verticillioides* and showed that fungi survived a dosage of 2.5 and 5 kGy while 10 kGy was lethal. FB1 production, as

Fig. 5 Effect of gamma irradiation on the viable count of *F. proliferatum*

Table 2 Effect of γ -irradiation on growth of *F. proliferatum* and FB1 biosynthesis

Dosage of treatment (kGy)	<i>F. proliferatum</i> growth after treatment (cm)		Fumonisin biosynthesis ($\mu\text{g/g}$ feed)
	5 days	10 days	
Control	4.9 $\pm$ 0.17	8.54 $\pm$ 0.20	5.0
2.5	3.8 $\pm$ 0.30	6.40 $\pm$ 0.55	11.0
5.0	NG	4.34 $\pm$ 0.45	6.75
7.5	NG	NG	ND
10.0	NG	NG	ND

Mean $\pm$ standard deviation, NG no growth, ND not detected

analyzed after 21 days of incubation was found to be influenced by irradiation (Table 2). The non-irradiated control feed had an FB1 content of 5 $\mu\text{g/g}$ of feed. Interestingly, the FB1 production in the feed sample treated with 2.5 kGy was more (11 $\mu\text{g/g}$ of feed) when compared to the control but the fungus viable count was less than that of the control. Production of FB1 was completely inactivated at 7.5 and 10 kGy doses, and the samples showed no fungal growth after 21 days of incubation. The stress created by low-dosage irradiation could probably have resulted in high FB1 production by *F. proliferatum*. Sreenivasa et al. (2009) reported that irradiation dosage of 7.5 and 10 kGy was efficient in controlling *Fusarium* contamination in maize and sorghum samples even after 90 days of storage. Another study by Mansur et al. (2014) showed that growth of *F. verticillioides* and fumonisin biosynthesis in maize decreased on exposure to higher doses (≥ 5 kGy) of irradiation, and a complete inactivation of the fungus required 30 kGy.

Conclusion

Here, we report high incidence of toxigenic *Fusarium* species, contaminating almost half of the feed samples analyzed. It is notable that irradiated feeds among our collection of samples also had *Fusarium* contamination. Γ -irradiation at doses of 7.5 kGy and above effectively inhibited the growth of *F. proliferatum* and FB1 production in feeds. The study underscores the importance of proper storage structures and suitable management of feed samples after irradiation to effectively reduce fungal contamination and improve the quality and shelf-life of feeds with respect to microbial safety. Also, proper irradiation time and dosage of the food stuffs and feeds is a must to ensure good management practice.

Acknowledgements Deepthi BV acknowledges University Grants Commission for the award of RGNF research fellowship. The authors also thank the Central Instrumentation Facility, the Institute of Excellence (IOE), and the University of Mysore for providing timely assistance in LC/MS analysis. We also thank Dr. Nagaraja H. for his kind effort in collecting feed samples from different districts of Karnataka, India.

Funding This research did not receive any specific Grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest The authors declare that no conflict of interest exists.

References

- Aziz NH, Souzan RM, Shahin AA (2006) Effect of gamma-irradiation on the occurrence of pathogenic microorganisms and nutritive value of four principal cereal grains. *Appl Radiat Isot* 64:1555–1562
- Bryden WL (2012) Food and feed, mycotoxins and the perpetual pentagram in a changing animal production environment. *Anim Prod Sci* 52:383–397
- Commission European (2007) Commission regulation 1126/2007 of 28 september 2007 amending regulation 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. *Off J Eur Union L* 255:14–17
- Dass RS, Sreenivasa MY, Janardhana GR (2007) High incidence of *Fusarium verticillioides* in animal and poultry feed mixtures produced in Karnataka, India. *Plant Pathol J* 6:174–178
- Deepa N, Nagaraja H, Sreenivasa MY (2016) Prevalence of fumonisin producing *Fusarium verticillioides* associated with cereal grown in Karnataka (India). *Food Sci Hum Wellness* 5:156–162
- Ferreira-castro FL, Aquino S, Greiner R, Ribeiro DHB, Reis TA, Correa B (2007) Effects of gamma radiation on maize samples contaminated with *Fusarium verticillioides*. *Appl Radiat Isot* 65:927–933
- Food and Drug Administration (2001) Guidance for industry: fumonisin levels in human foods and animal feeds; Final Guidance

- Food and Drug Administration (2011) FDA Mycotoxin Regulatory Guidance: a guide for grain elevators, feed manufacturers, grain processors and exporters
- Glenn AE (2007) Mycotoxigenic *Fusarium* species in animal feed. *Anim Feed Sci Tech* 137:213–240
- Grolichova M, Dvorak P, Musilova H (2004) Employing ionizing radiation to enhance food safety—A review. *Acta Vet Brno* 73:143–149
- Hollinger K, Ekperigin HE (1999) Mycotoxicosis in food producing animals. *Vet Clin N Am* 15:133–165
- Jindal N, Mahipal SK, Rottinghaus GE (1999) Occurrence of fumonisin B1 in maize and poultry feeds in Haryana, India. *Mycopathologia* 148:37–40
- Jurado M, Vazquez C, Marin S, Sanchis V, Gonzalez-Jaen MT (2006) PCR-based strategy to detect contamination with mycotoxigenic *Fusarium* species in maize. *Syst Appl Microbiol* 29:681–689
- Labuda R, Tancinova D, Hudec K (2003) Identification and enumeration of *Fusarium* species in poultry feed mixtures from Slovakia. *Ann Agric Environ Med* 10:61–66
- Leslie JF, Summerell BA (2006) The *Fusarium* laboratory manual. Blackwell Publishing, Iowa, USA, pp 224–227
- Mansur AR, Yu CC, Oh DH (2014) Efficiency of gamma irradiation to inactivate growth and fumonisin production of *Fusarium moniliforme* on corn grains. *J Microbiol Biotechnol* 24:209–216
- Marasas WFO (1995) Fumonisin: their implications for human and animal health. *Nat Toxins* 3:193–198
- Monge MP, Magnoli CE, Chiacchiera SM (2012) Survey of *Aspergillus* and *Fusarium* species and their mycotoxins in raw materials and poultry feeds from Córdoba, Argentina. *Mycotoxin Res* 28:111–122
- Nelson PE, Desjardins AE, Plattner RD (1993) Fumonisin, mycotoxins produced by *Fusarium* species: biology, chemistry and significance. *Annu Rev Phytopathol* 31:233–252
- Pacin AM, Gonzalez HHL, Etcheverry M, Resnik SL, Vivas L, Espin S (2003) Fungi associated with food and feed commodities from Ecuador. *Mycopathologia* 156:87–92
- Pereyra CM, Cavaglieri LR, Chiacchiera SM, Dalcero AM (2011) Mycobiota and mycotoxins contamination in raw materials and finished feed intended for fattening pigs production in eastern Argentina. *Vet Res Commun* 35:367–379
- Prathapkumar SH, Rao VS, Paramkishan RJ, Bhat RV (1997) Disease outbreak in laying hens arising from the consumption of fumonisin-contaminated food. *Brit Poultry Sci* 38:475–479
- Proctor RH, Plattner RD, Brown DW, Seo JA, Lee YW (2004) Discontinuous distribution of fumonisin biosynthetic genes in the *Gibberella fujikuroi* species complex. *Mycol Res* 108:815–822
- Sanchez-Rangel D, Sanjuan-Badillo A, Plasencia J (2005) Fumonisin produced by *Fusarium verticillioides* strains isolated from maize in Mexico and development of a polymerase chain reaction to detect potential toxigenic strains in grains. *J Agric Food Chem* 53:8565–8571
- Sreenivasa MY, Gonzalez-Jaen MT, Sharmila Dass R, Charith Raj AP, Janardhana GR (2008) A PCR-based assay for the detection and differentiation of potential fumonisin-producing *Fusarium verticillioides* isolated from Indian maize kernels. *Food Biotechnol* 22:160–170
- Sreenivasa MY, Maheshwar PK, Sanjay KR, Diwakar BT, Naidu KA, Janardhana GR (2009) Effect of gamma irradiation on the incidence and fumonisin production by *Fusarium* species occurring on maize and sorghum grains. *J Agric Technol* 5:325–335
- Wang J, Wang X, Zhou Y, Du L, Wang Q (2010) Fumonisin detection and analysis of potential fumonisin-producing *Fusarium* spp. in asparagus (*Asparagus officinalis* L.) in Zhejiang province of China. *J Sci Food Agric* 90:836–842
- World Health Organization (1999) High dose irradiation: wholesomeness of food irradiated with doses above 10 kGy. Report of a joint FAO/IAEA/WHO study group on high-dose. WHO Tech Rep Ser. pp 890–197