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MICROBIAL QUALITY EVALUATION OF VELVET BEAN SEEDS (*Mucuna pruriens* L. DC.) EXPOSED TO IONIZING RADIATION

[EVALUACIÓN MICROBIOLÓGICA DE SEMILLAS DE *Mucuna pruriens* L. DC. EXPUESTAS A RADIACIÓN IONIZANTE]

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SUMMARY

Seeds of *Mucuna* possess high nutraceutical value worldwide. We studied the occurrence of microflora, mycotoxins and textural features of seed samples of *Mucuna pruriens* before and after irradiation. Gamma irradiation of seeds (0, 2.5, 5, 7.5, 10, 15 and 30 kGy) resulted in dose-dependent decrease in microflora. Raw seeds harbored 12 fungal species capable of quality deterioration. Surface sterilized seeds revealed lower incidence of fungi than unsterilized seeds indicating them as surface contaminants. The most common fungal genera isolated belonged to *Aspergillus, Fusarium, Eurotium* and yeasts. Seeds consist of toxigenic moulds viz., *Aspergillus flavus, A. niger* and *Fusarium sp.* Both surface sterilized and unsterilized seeds showed total elimination of fungi at 10 kGy. Percent co-occurrence of fungi also significantly decreased after irradiation. Aflatoxin B_1 and ochratoxin-A in raw seeds degraded or were reduced after irradiation at 10 kGy, in addition, irradiation resulted in improvement in seed textural features. Fungal contaminants of *Mucuna* seeds can be effectively decontaminated on employing gamma irradiation at 10 kGy for safety and improvement of shelf life.

Key words: *Mucuna pruriens*, mycoflora, aflatoxin, ochratoxin, gamma irradiation, decontamination, texture.

RESUMEN

Las semillas de *Mucuna* poseen un alto valor nutraceutico. Se estudió el occurencia de microflora, micotoxinas y características de textura de semillas de *Mucuna pruriens* antes y después de irradiación. La irradiación Gamma de las semillas (0, 2.5, 5, 7.5, 10, 15 y 30 kGy) resultó en un decremento dosis dependiente de la microflora. En las semillas crudas se encontraron 12 especies de hongos con capacidad para deteriorar la semilla. Las semillas esterilizadas a nivel superficie presentaron una menor incidencia de hongos, demostrando que estos son contaminantes de superficie. Los hongos aislados más comunes pertenecían a los géneros *Aspergillus, Fusarium, Eurotium* y levaduras. Se logró una eliminación total de los hongos a 10 kGy. La aflatoxina B_1 y ochratoxina-A en semillas crudas fue degradada o reducida despues de radiacion a 10 kGy, adicionalmente la irradiación produjó una mejora en la textura de la semilla. Se concluye que la contaminación con hongos puesde ser reducida en semillas de mucuna por medio del uso de de radiaccion gamma a 10 kGy por aspectos de seguridad y para incrementar vida de anaquel.

Palabras clave: *Mucuna pruriens*, micoflora, aflatoxina, ochratoxina, irradiación gamma, descontaminación, textura.

INTRODUCTION

The velvet bean, *Mucuna pruriens* (L) DC. (Family: Fabaceae) is one of the popular tropical legumes widespread in Southeast Asia, Africa and Latin America. Many varieties and accessions of *Mucuna* have great demand in food and pharmaceutical industries. Nutritional importance of *Mucuna* seeds as a source of protein supplement in food and feed has been well documented (Siddhuraju *et al*., 2000; Siddhuraju and Becker, 2001; Bressani, 2002). They are used as thickening agents, beverages and in preparation of several food recipes (Haq, 1983; Wanjekeche *et al*., 2003). Tribals in Africa use roasted and finely powdered dry seeds as a supplement of coffee. Beyond nutritional qualities, *Mucuna* seeds possess high pharmaceutical value as raw drug material and are extensively used in indigenous ayurvedic medicine (Shaw and Bera, 1993; Prakash and Tewari, 1999). The L-DOPA extracted from

Mucuna seeds is used to provide symptomatic relief in Parkinson's disease (Nagashayana *et al*., 2000). These seeds are prescribed in the form of powder to treat leucorrhoea, spermatorrhoea and in cases requiring aphrodisiac action (Nadkarni, 1982). A variety of preparations made from *Mucuna* seeds are used for the management of several free radical-mediated diseases like ageing, rheumatoid arthritis, diabetes, male infertility and nervous disorders. *Mucuna* seeds have also been shown to be rich in antioxidant properties (Tripathi and Upadhyay, 2001). Although *Mucuna* seeds have versatile food and pharmaceutical values, methods of processing and storing in tropical conditions render the seeds susceptible to microbial contamination including toxigenic moulds incurring substantial losses. Fungal and mycotoxin contamination is of main concern to minimize the economic losses and reduce the potential risks to human and livestock health (Ueno, 2000). Recently, the loss in worlds food supply has been estimated to be about 25% due to microbial contamination, improper handling and storage. In addition, ban on the use of chemical fumigants (e.g. methyl bromide, ethylene dioxide) for food preservation in developed countries (2005) as well as in developing countries (2015) (Anonymous, 1995) has forced to depend on new approaches of physical methods of preservation. Irradiation offers an effective alternative to chemical fumigants, which are either banned or of restricted usage due to environmental, health and safety concerns. Radiation processing by employing gamma rays has been established as a safe and effective physical means for microbial decontamination and to extend the shelf life of food and agricultural commodities (Diehl, 1990; Wilkinson and Gould, 1998; FAO/IAEA/WHO, 1999). Currently, gamma irradiation has been employed as a safe method of preservation of food and agricultural commodities in pilot as well as large scale (ICGFI, 1999). To date, no detailed reports are available on the extent of microbial and toxin contamination of *Mucuna* seeds. Thus, the main objective of the current study was to evaluate qualitatively and quantitatively the rate of microbial contaminants, mycotoxins (aflatoxin and ochratoxin) and the textural features before and after gamma irradiation to provide baseline data for further commercialization of radiation technology for safety and quality purposes.

MATERIALS AND METHODS

Seed Samples

Fresh, dried *Mucuna* seeds from a single lot, weighing about 20 kg were procured from a leading ayurvedic dealer of Kerala, Southern India. Clean seeds with no apparent physical damage or insect infestation were selected for the study. Samples were subdivided as per the requirements discussed below to analyze different parameters.

Moisture and water activity

Moisture content of the seeds was determined gravimetrically on oven drying (105±1ºC, 16±1 hr) until a constant weight was attained. The difference in the initial and final weight of the samples was expressed as percent moisture. Water activity (a_w) of the samples was measured using water activity meter (Rotronic Hygropalm, Switzerland) and the measurements were carried out to an accuracy of 0.005 units at 24ºC.

Gamma irradiation

Mucuna seeds (~50 g) packed in specially designed biaxially oriented polypropylene bags (BOPP, 25 µm) were exposed to gamma irradiation doses (2.5, 5.0, 7.5, 10, 15 and 30 kGy) at room temperature (25 ± 1ºC) in a ^{60}Co package irradiator (ISOMED, Bhabha Atomic Research Centre, BARC, Trombay, Mumbai, India). The dose rate of the irradiator was 6.5 kGy/h (Fricke and Hart, 1966). Non-irradiated samples packed as described above served as control (0 kGy).

Microbial load

Total surface microbial load in colony forming units (cfu) was determined by the standard spread-plate method. Both the irradiated and non-irradiated samples were suspended in sterile saline (NaCl, 0.85%), homogenized, serially diluted and plated on nutrient agar (bacteria), Kushtor Knight agar (actinomycetes) and Czapek Dox Agar (CZA) (fungi and yeasts). For determination of *Escherichia coli*, Eosine Methylene Blue agar was employed (incubation, 37.5ºC). Each sample was analyzed in five replicates.

Mycological studies

Isolation and identification of fungi

A total of 100 seeds was selected and separately plated without surface sterilization on CZA and Dichloron Glycerol 18 (DG-18) medium. The DG-18 medium was employed for better isolation of xerophilic moulds (Hocking and Pitt, 1980). Similar set was maintained, wherein the seeds were surface sterilized using sodium hypochlorite (1%, 1 min), rinsed thrice in sterile distilled water and plated. Plates were incubated (25ºC, 5-7 days) and examined for the occurrence of mycoflora and their percent incidence:

Incidence of fungi (%) = (Number of seeds on which a species is present) ÷ (Total number of seeds screened) × 100

Fungi isolated were subcultured and identified based on standard references (Pitt *et al.,* 1983, Klich and Pitt, 1992, Watanabe, 1994). All the synthetic media used in present investigation were procured from Hi-Media Laboratories Pvt. Ltd., Mumbai, India.

Mycotoxins

Aflatoxins (B_1, B_2, G_1 and G_2) were analyzed in raw and irradiated *Mucuna* seeds at standardized decontamination dose (10 kGy). Ten grams of finely ground seed flour (sub sample from 500 g flour) was taken in an Erlenmeyer flask (250 ml) and 100 ml mixture of methanol + water (8:2 v/v) was added. The slurry was shaken on a rotary shaker (30 min, 150 rpm) and filtered through Whatman # 1 filter paper. The filtrate was transferred to a conical flask (250 ml) and the interfering pigments were removed by Ferric Gel clean up procedure (Velasco, 1970). Later, aflatoxins were extracted from the filtrate on addition of equal volumes of chloroform. The chloroform layer was evaporated to dryness, re-dissolved in a known quantity of chloroform and toxins were analyzed by TLC (Silica Gel-60, Merck, Germany) (Sharma *et al.,* 1985). Quantification of toxins was done by TLC flurodensitometry (CS-9000, Shimadzu Corp., Kyoto, Japan, detection limit of 2 ng/g) (Hajare *et al.*, 2005).

Raw and irradiated *Mucuna* seeds (decontamination dose, 10 kGy) were analyzed for Ochratoxin A (OTA) by HPLC method. Extraction and purification of samples were carried out according to the method outlined by Patel *et al.* (1997) with a slight modification. Ten gram samples of seed powder were extracted in chloroform (50 ml) + orthophorphoric acid (0.1M, 5 ml) for 30 min on a mechanical shaker. Extract was filtered through Whatman # 1 filter paper and the filtrate was evaporated under vacuum in rotary evaporator (~ 40 ºC). Clean up was performed on silica gel column (60-120 mesh). Five gram of silica gel was slurred in chloroform and packed in a glass column (1.5×30 cm) with a stopper. After silica gel settled, one gram of anhydrous sodium sulfate was added, the residue was evaporated, re-dissolved in 2 ml chloroform twice, loaded on to silica gel column and drained by gravity. The column was flushed with chloroform : methanol (97:3) (50 ml) and washes were discarded. The OTA was eluted in toluene : acetic acid (9:1) (50 ml), evaporated under vacuum (40 ºC), transferred to 5 ml test tubes each containing toluene : acetic acid (9:1) and evaporated under nitrogen. The residue was dissolved in the HPLC solvent and the analysis was carried out (15 cm column, Bandaclone RPC-18 Spherisorb) using acetonitrile : water : acetic acid (55:45:2%) as mobile phase (flow rate, 1ml/min). The excitation maxima were 330 nm and emission at 460 nm. The OTA was detected and quantified by comparison of peak areas of samples and standard OTA. Presence of OTA was confirmed with Boron trifluride (BF3) reaction with sample extracts. A 50 ml of BF3 was added to dried extract, at 60 ºC for a few seconds, evaporated, dissolved in mobile phase and injected into HPLC column. The OTA peaks were confirmed by changes in the retention time in samples and standards.

Texture studies

The texture of *Mucuna* seeds was measured by texture analyzer instrument (TAHD plus) equipped with a fabricated cylindrical probe (stainless steel, 5 mm diameter; Stable Microsystems, Surrey, England). The texture was calculated as the force (kg) measuring resistance to the compression imposed by the cylindrical probe with the help of an inbuilt software programme. The instrument was calibrated before use and the parameters and compression for analysis include: pre-test speed, 10 mm/sec; test speed, 1 mm/sec; post-test speed, 10 mm/sec; target mode, distance (3.000 mm); trigger type, auto (force); trigger force, 15.0 (g) respectively.

Statistical analysis

For comparison of mycofloral profiles, student t-test was employed (Stat Soft Inc, 1995), while One-way ANOVA for comparison of moisture, a_w, toxins and texture (ORIGIN® version 6.0, Microcal software Inc., Northampton, MA 0 1060, USA).

RESULTS AND DISCUSSION

Moisture and water activity

Moisture and a_w of samples are of great significance in radiation processing as it controls the net radiochemical changes during irradiation and storage (Wilkinson and Gould, 1998). In our study, gamma irradiation significantly influenced the moisture and a_w between control and irradiated *Mucuna* seeds ($P < 0.05$) (Fig. 1). Moisture of seeds decreased on irradiation, which ranged between 9.97 (control) and 8.19 (30 kGy). Warchalewski *et al*. (1998) have attributed the decrease in moisture of samples to time and dose of irradiation. Significant decrease in a_w was recorded between control and irradiated seeds (control, 0.723; 30 kGy, 0.717) ($P < 0.05$). Such differences might be attributed to the biological variations in seeds prior to irradiation. Although, the observed moisture and a_w of seeds was low and safe for storage against mould attack, if the storage conditions are inadequate (e.g. fluctuations in temperature and humidity), water

can drift through the gas phase and result in increased a_w and thereby support mould growth leading to spoilage and decline in shelf life (Moss, 1999).

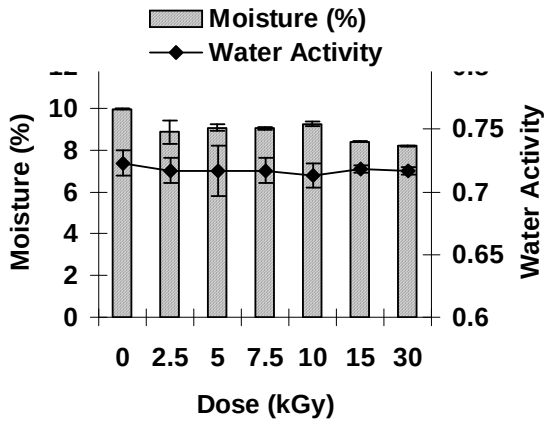

Figure 1. Changes in percent moisture and water activity of *Mucuna* seeds subjected to different doses of gamma irradiation (n=5, mean ± SD).

Microbial counts

Dose dependent decrease in microbial load was seen in *Mucuna* seeds exposed to gamma irradiation (Table 1). The total bacterial count decreased at doses of 2.5 and 5 kGy, while at 7.5 kGy no bacteria were recovered. Coliforms and actinomycetes were absent in raw as well as irradiated samples. Total fungal count was high in control samples (5.9×10^3), while at 7.5 kGy they were undetectable. Yeast population declined by 2-log cycle at 2.5 kGy, while no yeasts were seen at 5 kGy. At 5 and 7.5 kGy, microbial load reduced substantially compared to control samples. Thus, these doses might prove to be beneficial for surface disinfection of *Mucuna* seeds. Doses of 5 to 10 kGy is almost equivalent to commercial fumigation and doses of the order <10 kGy has been accepted as safe in terms of microbial safety of the product (Farkas, 1988, 1998; WHO, 1999).

Fungal incidence

Domsch *et al*. (1981) indicated that the mould contamination of food commodities was due to extraneous contamination by dust followed by storage at humid conditions. Legume seeds are prone for fungal contamination resulting in the production of toxic secondary metabolites, which cause severe health disorders (D'Mello and MacDonald, 1997; Peraica *et al*., 1999; Ahmad and Singh, 1991; Pitt, 2000). If these contaminant moulds are not eliminated, on consumption they may pose serious health risks for humans and livestock. Feed-borne fungal contamination has been shown to affect the human and livestock health through animal products (e.g. milk, eggs, meat) (Bastianelli and Le Bas, 2000; Chhabra and Singh, 2005). There are sufficient evidences of human liver cancer (Groopman *et al*., 1988; Massey *et al*., 1995) and Kwashiorkar disease (protein-energy malnutrition in children) (Adhikari *et al*., 1994) on consumption of mould contaminated food. Similarly, consumption of contaminated feeds has been shown to incur heavy loss and lower the meat quality in farm animals (Bonomi *et al*., 1994). Hence, complete elimination of colonized moulds in plant produce assumes importance when used for consumption purpose.

Although visual examination of *Mucuna* seeds reveals mould-free, a wide range of fungi was recovered on plating. Fungal incidence of *Mucuna* seeds on CZA and DG-18 are projected in Tables 2 and 3. A total of 12 species of fungi isolated belonged to the genera *Absidia, Aspergillus, Cladosporium, Eurotium, Fusarium, Mucor, Rhizopus* and yeast. The genus *Aspergillus* comprised of six species *viz*., *A. flavus, A. fumigatus A. niger, A. ochraceus, A. parasiticus* and *A. tamarii*. Fungal incidence varied between control and surface sterilized seeds, media and doses of irradiation.

Table 1. Total microbial counts of control and gamma irradiated *Mucuna* seeds ($\log_{10}$ Cfu/g) (n=5, mean ± SD).

Microbe	Dose (kGy)*				
	0	2.5	5.0	7.5	10
Bacteria	$1.5 \pm 0.2 \times 10^2$	$4.0 \pm 0.0 \times 10^1$	$3.0 \pm 0.1 \times 10^1$	<10	<10
Escherichia coli	0	0	0	0	0
Actinomyecetes	0	0	0	0	0
Fungi	$5.9 \pm 0.35 \times 10^3$	$3.1 \pm 0.2 \times 10^3$	$2.1 \pm 0.2 \times 10^2$	<10	<10
Yeast	$3.3 \pm 0.2 \times 10^4$	$1.2 \pm 0.4 \times 10^2$	<10	<10	<10

*No microorganisms were present at doses 15 and 30 kGy

Table 2. Percent incidence of fungi in control and gamma irradiated *Mucuna* seeds on DG-18 (US, unsterilized; S, sterilized)

Fungus	Dose (kGy)*				
	0	2.5	5	7.5	10
	US/S	US/S	US/S	US/S	US/S
Absidia Van Tieghem	5/5	5/5	5/0	0/0	0/0
Aspergillus flavus Link.	86/24	76/10	33/10	19/0	0/0
A. niger Van Tieghem	67/24	62/19	38/10	19/10	0/0
A. tamarii Kita	0/0	0/0	5/0	0/0	0/0
Cladosporium sphaerospermum Penz.	0/5	0/10	0/10	0/0	0/0
Eurotium amstelodami Mangin	0/67	0/48	0/0	0/0	0/0
Eurotium rubrum Kőnig, Spieckermann *et* Bremer	81/81	81/67	80/5	48/5	0/0
Fusarium sp.	67/14	5/5	5/5	5/10	0/0
Mucor sp.	33/5	0/5	0/0	0/0	0/0
Penicillium sp. (white)	5/0	5/0	5/0	0/0	0/0
Rhizopus sp.	14/5	19/0	0/0	0/0	0/0
Yeasts (white to pinkish)	0/19	5/14	5/5	0/5	1/0

*No microorganisms were recovered at doses 15 and 30 kGy

Table 3. Percent incidence of fungi in control and gamma irradiated *Mucuna* seeds on CZA medium (US, unsterilized; S, sterilized)

Fungus	Dose (kGy)				
	0	2.5	5	7.5	10
	US/S	US/S	US/S	US/S	US/S
Aspergillus flavus Link.	52/24	10/19	5/0	0/0	0/0
A. fumigatus Fres.	21/0	0/0	0/0	0/0	0/0
A. niger Van Tieghem	90/24	57/5	10/0	0/0	0/0
A. ochraceus Wilhelm	19/19	10/19	5/0	0/0	0/0
A. parasiticus Speare	5/0	14/0	10/0	0/0	0/0
Cladosporium sphaerospermum Penz.	57/5	24/5	5/0	5/0	0/0
Eurotium rubrum Kőnig, Spieckermann *et* Bremer	48/0	48/0	48/0	29/0	0/0
Fusarium sp.	90/71	29/33	24/5	19/5	0/0
Mucor sp.	5/0	0/0	0/0	0/0	0/0
Rhizopus sp.	71/62	28/33	0/0	0/0	0/0
Trichoderma harzianum Rifai	5/0	0/0	0/0	0/0	0/0
Sterile isolate (non sporulating mycelia)	5/0	0/0	0/0	0/0	0/0
Yeasts (white to pinkish)	50/38	48/33	48/17	19/14	2/0

*No microorganisms were recovered at 15 and 30 kGy dose

Examination of control and surface sterilized seeds revealed superficial as well as internal fungal contamination. Irrespective of the media used (CZA and DG-18), control seeds showed higher fungal incidence. Unsterilized seeds on DG-18 showed high frequencies of *A. flavus* (86%), *Eurotium* sp. (81%), *A. niger* (67%) and *Fusarium* sp. (67%), while it was *A. niger* (90%) and *Fusarium* sp. (90%) on CZA. A marked difference observed in CZA was the occurrence of *A. ochraceus* (19%) on extended period of incubation of seeds (9-10 days). This may possibly be due to fungal succession (Wicklow, 1995), wherein the colonized moulds create microenvironments through their metabolic activities leading to increased moisture and temperature suitable for growth of less xerophilic moulds. Interspecific interactions might have also played a significant role as each fungus prefers specific a_w for growth and expression (Frisvad, 1995; Marin *et al*., 1998). Moulds are more tolerant to low a_w than yeasts and bacteria (Leistner, *et al*., 1981; Beuchat, 1987; Lenovich, 1987). Incidence of *Aspergillus* spp. in *Mucuna* seeds at low a_w indicates their xerophilic nature, while dominance at higher a_w might be due to their competitive ability. Interestingly, higher number of fungi was encountered in CZA than DG-18 with an exceptional isolation of *A. fumigatus*

(21%), *A. parasiticus* (5%), *Trichoderma harzianum* (5%) and sterile isolate (non-sporulating mycelia; 5%). The percent occurrence of *Penicillum* sp., a major post-harvest storage fungus, was low in our study, which may be attributed to the negative correlation with *Aspergillus* sp. and *Rhizopus* sp. rendering the substrate less suitable for colonization as predicted by Purcell *et al*. (1980).

Irrespective of the media used, fungal incidence in surface sterilized seeds was lower than unsterilized seeds ($P < 0.001$) indicating the presence of contaminants on the surface (see Table 2 and 3). This difference may be ascribed to the fact that *Mucuna* seeds are usually spread on soil or on open floors for sun drying. Incidence of *Cladosporium*, *Fusarium* and *Trichoderma* in *Mucuna* seeds indicates contamination or invasion in field during pre-harvest period. The storage environment and place of collection of seeds might contribute substantially for microbial contamination. For instance, in the tropical and subtropical environments relatively high humidity and ambient temperature prevail and usually the number of fungal isolates will be more than other geographical regions (Lopez and Christensen, 1970; Aziz *et al*., 1998; Bhat and Vasanthi, 2003). Gamma irradiation of *Mucuna* seeds showed a dose-dependent decline in the total mould incidence. All irradiation doses employed irrespective of unsterilized or sterilized seeds showed significant decrease in fungal infection against control (P=0.0006-0.03) on incubation in CZA and DG-18 (P=0.009-0.04).

Toxigenic moulds (*A. flavus*, *A. niger* and *Fusarium* sp.) decreased up to 50% at 5 kGy. However, 10 kGy was more effective and can be considered as an optimal dose for fungal decontamination of *Mucuna* seeds. Figure 2 projects the pattern of decline in the total fungal population and toxigenic moulds of *Mucuna* seeds exposed to gamma irradiation. At higher doses (15 and 30 kGy), no microorganisms survived and these can be established as sterilization doses for *Mucuna* seeds although there is no necessity to employ this in practice.

The impact of gamma irradiation on fungal incidence of *Mucuna* seeds has not been hitherto investigated. Many reports are available on application of gamma irradiation as a safe and effective mean to decontaminate economically valued products. A dose of the order 5-6 kGy is sufficient to inactivate the growth of filamentous fungi (Diehl, 1990; Aziz *et al*., 1997 a, b; Dogbevi *et al*., 2000). A single gamma irradiation treatment with doses of the order <10 kGy has been accepted as safe and effective for microbial safety of the product without affecting the sensory, nutritional and technical qualities (WHO 1994, 1999; Farkas, 1998). Chiou (1994) has reported the efficacy of gamma irradiation (2.5 kGy) on fungal contamination of peanut kernels. Similarly, 1 kGy effectively inhibited the fungal growth in peanuts (Hilmy *et al*., 1995). As irradiated food and agricultural commodities can be stored for long term without refrigeration in tropical conditions (IAEA, 1995), radiation processing by gamma rays may prove to be beneficial in decontamination of *Mucuna* seeds.

Co-occurrence of fungi

Generally, fungal co-occurrence in any seeds indicates the possible competition and succession or antagonism among colonized fungi. Irrespective of the media used, the percent co-occurrence of toxigenic moulds (*A. flavus*, *A. niger* and *Fusarium* sp.) with other fungi was high in control than irradiated seeds. Irrespective of sterilized or non-sterilized seeds, no fungal co-occurrence was seen in 5 kGy (CZA) or 10 kGy (DG-18). Co-occurrence of toxigenic moulds with other fungi was high in unsterilized control seeds in CZA, while it was sterilized seeds in DG-18. The most common combination of co-occurrence in unsterilized control seeds in CZA include: *A. niger* + *Fusarium* sp. (100%), *A. niger* + *A. ochraceus* + *Fusarium* sp. (19%), *Fusarium* sp.+ *A. parasitic*us (14%) and *A. niger* + *Fusarium* sp. + *Mucor* sp. (10%), while in sterilized control seeds it was *A. niger* + *Fusarium* sp. (33%), *A. niger* + yeast (33%), *A. niger* + *A. ochraceus* (19%) and *Fusarium* sp. + *Mucor* sp. (19%). In DG-18, unsterilized control seeds consist of *A. flavus* + *Mucor* sp. (43%), *Fusarium* sp. + *Eurotium* sp. (24%) and *A. ni*ger + *Fusarium* sp. (19%), while in sterilized control seeds it was *A. flavus* + *Eurotium* sp. (43%), *A. fla*vus + *Fusarium* sp. (28%) and *A. niger* + *Fusarium* sp. (14%). Percent co-occurrence decreased between control and irradiated seeds supporting the impact of gamma irradiation on fungal co-occurrence.

Fungal co-occurrence has been shown to inhibit or reduce the toxin concentration in substrate (Mann and Rehm 1976). Coinoculation of *A. niger* and *T. viride* on to maize or peanuts contaminated with *A. flavus* showed reduction in aflatoxin production (Wicklow *et al*., 1980; Aziz and Shahin, 1997). Frequency of occurrence of *A. flavus* on almonds also declined on coinoculation with *Ulocladium charatum* (Phillips *et al*., 1979). Results of co-occurrence in the current study might help to design strategies to prevent the growth of toxigenic moulds through fungal coinoculation at a minimal dose of gamma irradiation.

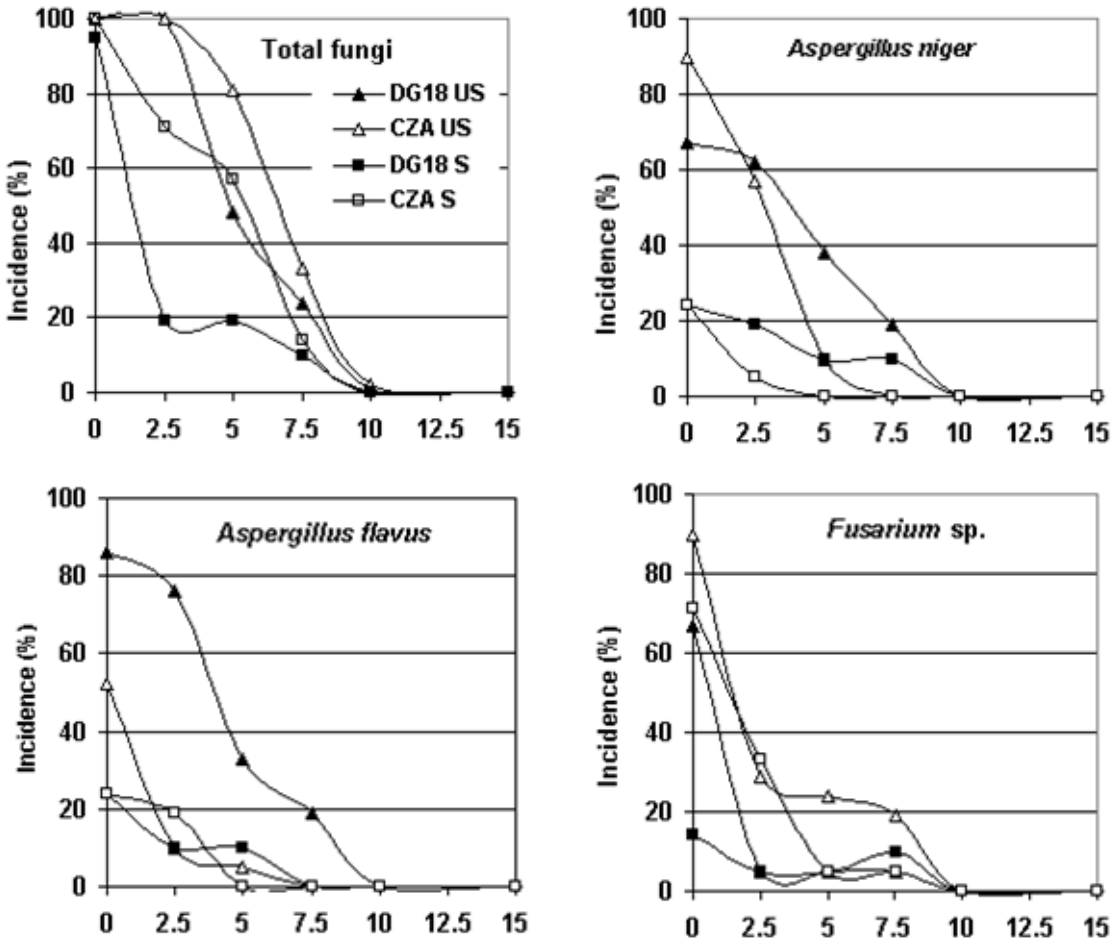

Figure 2. Percent incidence of total fungi and toxigenic fungi (*Aspergillus flavus, A. niger* and *Fusarium* sp.) in *Mucuna* seeds subjected to different doses of gamma irradiation (media: DG-18, CZA; US, unsterilized; S, sterilized).

Mycotoxins

In the current study, aflatoxin B_1 was detected only in control *Mucuna* seeds (28.5 ± 0.01 ng/g). At the standardized irradiation dose (10 kGy) it was below the detectable limit indicating its possible degradation. This dose assumes importance as it is most efficient dose of decontamination of *Mucuna* seeds. Similarly, doses of the order up to 10 kGy have been generally recommended for total elimination of fungi. Irradiation up to this dose is safe, nutritionally acceptable and requires no toxicological evaluation (Diehl, 1990; WHO, 1994, Wilkinson and Gould, 1998). Inhibition of *A. flavus* and production of aflatoxin B_1 have been reported by Aziz and Mahrous (2004) at 5 kGy irradiation in crop seeds (wheat, soyabean and fababean). Complete degradation of aflatoxin B_1 at 10 kGy was evident in peanut meal (Temcharoen and Thilly, 1982). Chipley and Uraih (1980) have also demonstrated a reduction in aflatoxin by gamma irradiation (5-10 M-rad). Our observations corroborate with the above reports and support the efficacy of gamma irradiation in degradation of aflatoxins. Rushtom (1997) has attributed the degradation of

aflatoxin by irradiation to the presence of water content, wherein radiolysis of water leads to the formation of free radicals, which in turn destabilizes the furan ring of aflatoxin B_1 giving rise to products of lower biological activity. Co-occurence of fungi in *Mucuna* seeds might have also played a significant role in degradation of aflatoxin. Usually, growth and activity of a specific fungus is dependent on the colonization and biological properties of co-invading fungus (or fungi) in degradation of aflatoxins. For instance, *A. niger*, a strong antagonist of *A. flavus* has been predicted to restrict the synthesis of aflatoxins (Wicklow *et al*., 1980; Roy and Chourasia, 1990), which is evident in our study too.

Some of the common fungi producing ochratoxin (OTA) are *A. carbonarius*, *A. niger*, *A. ochraceus* and *Penicillium* spp. (Moss, 1996). OTA is known for nephrotoxic, teratogenic, carcinogenic and immunotoxic effects (IARC, 1993; Holer, 1998; Bayman *et al*., 2002). The provisional daily intake of OTA in foods is extremely low (1-16 ng/kg body weight) (Codex, 1998). *Aspergillus niger* and *A. ochraceus* might have produced OTA in *Mucuna* seeds. The OTA content showed significant decrease ($P < 0.05$) between control (4.8 ± 0.1 ppb) and irradiated seeds (10 kGy, 3.2 ± 0.3 ppb). However, OTA could not be totally degraded in *Mucuna* seeds even at 10 kGy indicating its stability. Presence of OTA in *Mucuna* seeds might have occurred during pre-harvest stage and the concentration and potential toxigenicity might have increased during post-harvest storage. Further exploration is necessary to find out the exact phase wherein seeds are vulnerable for ochratoxin production.

Texture

The force required to compress the seeds help to evaluate the hardness and those in turn overall qualities. The texture of *Mucuna* seeds decreased from 45.41 N (control) up to 39.07 N (30 kGy) (Fig. 3). Except for the seeds irradiated at 2.5 and 5 kGy ($P > 0.05$), dose dependent significant decrease in texture/firmness was seen ($P < 0.05$) against control. Irradiation results in softening of plant tissues due to degradation of polymers (e.g. pectin and cellulose) and solubilization of cell wall polysaccharides or pectic substances (Glegg and Kertesz, 1956, 1957; Somogyi and Romani, 1964; Bartley and Knee, 1982; Howard and Buescher, 1989; D'Amour *et al*., 1993). Such textural changes affect cell membrane and leads to the loss of intracellular moisture and cell turgescence (Voisine *et al*., 1993). The hardness of seed has positive correlation with cooking time and the seeds having least hardness/thickness are usually advocated for consumption. Decrease in texture/firmness of irradiated *Mucuna* seeds in the current study may be advantageous to minimize duration of cooking. However, loss of texture will make any plant produce susceptible for re-infection/diseases by storage moulds requiring low a_w for growth and proliferation. Therefore, care should be exercised to maintain the packed irradiated products at minimal environmental fluctuations.

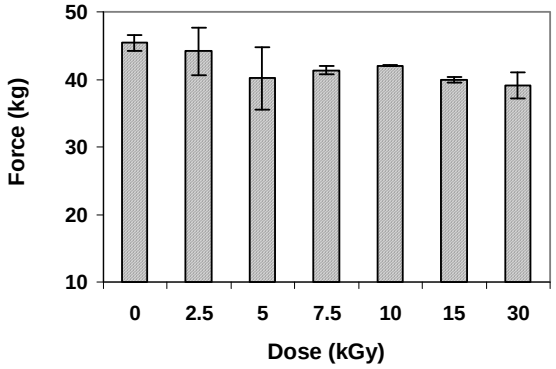

Figure 3. Textural changes of *Mucuna* seeds subjected to different doses of gamma irradiation (n=5, mean ± SD).

CONCLUSIONS

Mucuna seeds hold high promise as a nutraceutically valued product and meet the requirements of protein-energy malnutrition in developing countries. Results of the present study revealed that *Mucuna* seeds harbour a wide variety of moulds with aflatoxin and ochratoxin contamination. Either consumption or product preparation made out of such contaminated seeds may pose health risks. Gamma irradiation of *Mucuna* seeds at 10 kGy has been suggested as an effective dose for decontamination. Irradiation has also been proved to be efficient in improving the textural features of *Mucuna* seeds. Further investigations should emphasize the possible changes in the nutritional and antinutritional properties upon gamma irradiation. However, the practicality of employing radiation technology for safe storage will be successful, once the consumers know the benefits and advantages over other means of preservation. Thus, researchers, government and non-government agencies need to popularize this technology and its importance. Irradiated *Mucuna* seeds can be effectively marketed by displaying a logo indicating "free from toxic fumigants''.

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