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Effect of gamma radiation on seed mycoflora of sunflower at different storage periods

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Abstract
The effect of gamma radiation @ 0.1, 0.3, 0.5, 1.0, 1.5 and 2.0 kGy on seed mycoflora of sunflower at different storage periods (upto 3 months) was studied. A total of 16 seedborne fungi belonging to 13 genera *viz*., *Alternaria* sp., *Macrophomina phaseolina, Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Aspergillus ustus, Emericella nidulans, Fusarium* sp., *Epicoccum* sp., *Cladosporium* sp., *Curvularia* sp., *Chaetomium* sp., *Drechslera* sp., *Rhizopus* sp., *Trichoderma* sp. and *Penicillium* sp. were recovered from untreated and treated seeds at different storage periods. Of the treatments used, gamma radiation at 2.0 kGy (25.56%) was found significantly superior to all other treatments followed by 1.5 kGy (40.41%) and the least effective (73.34%) was 0.1 kGy. The per cent seed infection by different seed mycoflora increased with the increase in storage period. However, there was a gradual decline in field mycoflora *viz*., *Alternaria* sp., *Macrophomina phaseolina, Fusarium* sp. and *Drechslera* sp. and gradual increase in storage mycoflora *viz*., *Aspergillus flavus, Aspergillus niger, Cladosporium* sp., *Curvularia* sp. etc. was found with the increase in storage period. Effect of gamma radiation on sunflower seed quality parameters was also tested and results indicated that gamma radiation can't be used as seed treatment in sunflower.

Keywords: Sunflower seed mycoflora, gamma radiation seed treatments, storage mycoflora, standard blotter method

Introduction
Sunflower (*Helianthus annuus* L.) is one of the most popular oilseed crops grown in India. Sunflower seeds contain 40-50% oil, 23% of protein and constitute excellent source of unsaturated fats, fiber, linoleic acid and important nutrients, selenium, copper, zinc, vitamin E and B complex as well (Afzal *et al*., 2010) [1]. The total area of sunflower in India is 0.69 Mha with a production of 0.50Mt (Indiastat, 2013-14) [8]. Karnataka and Andhra Pradesh are the major sunflower growing states in India.

Seed health plays an important role in successful cultivation and yield exploration of a crop. Fungi are the main component of microflora associated with seeds and are the main cause of deterioration and loss observed during storage (Tanaka *et al.*, 2001) [16]. The associated microorganisms may be pathogenic or non-pathogenic in nature. Major seedborne diseases of sunflower include, leaf blight (*Alternaria helianthi*), head rot (*Rhizopus arrhizus*), collar rot (*Sclerotium rolfsii*) and downy mildew (*Plasmopara halstedii*). It was reported that, 20-30 per cent loss in germinability of sunflower was due to seedborne diseases (Jamaria *et al.*, 1975) [10]. Therefore, management of seedborne fungi is extremely important for realization of full yield potential of cultivars.

Though fungicides have played an important role in increasing production and management of diseases, their indiscriminate use has led to several problems such as development of resistance in fungi to fungicides, destruction of beneficial organisms and direct and indirect influence on human health. Thus, exploration of other alternative disease management options need to be considered.

Ionizing irradiation technique is a physical process that appears promising for the control of microorganisms especially insects and fungi in grains and seeds during storage, (Toledo *et al.*, 2007) [17]. Gamma irradiation of food in order to reduce natural or pathogenic microflora and increase the shelf life of products (spices, grains, meat, fruits and vegetables) is one of the technologies that are growing around the world. Further, gamma radiation was found to be beneficial at low levels (1-2 kGy) in reducing the mycoflora of treated seed without affecting the seed quality parameters. Higher dosages resulted in negative effects on seed quality

parameters even though seed mycoflora was reduced (Maity *et al.*, 2009) [12]. Recently, it was also reported that, gamma rays influence plant growth and development by inducing cytological, genetical, biochemical and physiological changes in cells and tissues (Aparna *et al.*, 2014) [2].

In the present study, effect of gamma radiation at different dosages on sunflower seed mycoflora was evaluated over a period of three months of storage after seed treatment.

Material and methods

Seeds of sunflower hybrid DRSH-1 were collected from IIOR, Rajendranagar, Hyderabad and stored at ambient storage temperature of 28 ± 2^0C. This experiment was conducted at SRTC, Rajendranagar, Hyderabad.

Sunflower seeds were irradiated in continuous gamma sterilization plant (GC 5000) with an initial activity of 444 TBq (12000Ci) and Cobalt-60 (1.61 kGy hr^{-1}) as source at Quality Control Laboratory, Rajendranagar, Hyderabad. Seeds for irradiation were placed in an irradiation chamber located in vertical drawer inside the Lead flask. Radiation field was provided by a set of stationary Cobalt-60 source placed in a cylindrical cage. The Lead shield provided around the source was adequate to keep the external radiation field well within permissible limits. The seeds were irradiated for 0.1, 0.3, 0.5, 1.0, 1.5 and 2.0 kGy and were stored in butter paper bags along with chemical (Carbendazim - 0.2%) and untreated control for further use.

The effect of gamma irradiation on seed mycoflora was assessed by employing standard blotter method (ISTA, 1996) [9]. The randomly selected 400 treated seeds were subjected to seed health testing at different intervals *viz*., immediately after treatment, one day after treatment, one week after treatment, two weeks after treatment, three weeks after treatment, one month after treatment, two months after treatment and three months after treatment consecutively along with controls. Seeds treated with a standard seed dressing fungicide carbendazim and untreated seeds were served as controls. The data on number of seeds infected by different fungi and a specific fungus was recorded separately to calculate per cent seed infection and frequency of a specific fungus.

Detection of seed mycoflora by standard blotter method

Sterilized blotting paper discs of 90mm diameter were placed in sterile Petri plates and moistened with sterile distilled water. The excess water was drained off from the plates. Seeds were transferred to the plates containing moist blotting paper discs. Ten seeds per plate were placed at equidistance, 10 such plates were maintained under each replication. The experiment was conducted with four replications and under each replication hundred seeds were tested. The plates were incubated at 24 ± 2^0C for seven days in an incubator. The mycoflora observed on seeds were isolated and identified.

Data recording

On 8th day, the incubated seeds were examined under stereo binocular microscope. The mycelium and the fungal structures obtained from the seeds were further observed critically under 10x and then under 40x objective lens of a compound microscope by preparing water mount slides.

Data on number of seeds infected by different fungi and a specific fungus were recorded separately to calculate per cent seed infection and frequency respectively. To calculate per cent seed infection (Aslam *et al.*, 2015) [3] and frequency of the species (Neha and Razia, 2013) [14] the following formulae were used.

$$\text{Per cent seed infection} = \frac{\text{Number of infected seeds}}{\text{Total number of seeds}} \times 100$$

$$\text{Frequency} = \frac{\text{No. of seeds containing a specific fungus}}{\text{Total number of seeds}} \times 100$$

Isolation of Fungi

Fungal colonies or sporulating structures obtained from seeds after incubation through both the methods were isolated separately onto fresh PDA medium in Petri plates. Pure cultures of the fungi isolated were obtained by adopting hyphal tip method or single spore isolation technique (Tuite, 1969) [18]. Pure cultures thus obtained were maintained on PDA slants.

Identification of Fungi

Identification of various seed mycoflora was done using relevant keys given by Subramanian (1971) [15], Booth (1971) [5], Barnett (1965) [4] and descriptions of CMI (1970) [6].

The data obtained was statistically analyzed using factorial CRD as per the procedures suggested by Gomez and Gomez (1984) [7].

Results and discussion

A total of 16 seedborne fungi belonging to 13 genera *viz*., *Alternaria* sp., *Macrophomina phaseolina, Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Aspergillus ustus, Emericella nidulans, Fusarium* sp., *Epicoccum* sp., *Cladosporium* sp., *Curvularia* sp., *Chaetomium* sp., *Drechslera* sp., *Rhizopus* sp., *Trichoderma* sp. and *Penicillium* sp. (Table 2) were recovered from untreated and treated seeds at different storage periods. It was observed that, the per cent seed infection by different seed mycoflora increased with the increase in storage period. However, there was a gradual decline in field mycoflora *viz., Alternaria* sp., *Macrophomina phaseolina, Fusarium* sp. and *Drechslera* sp. and gradual increase in storage mycoflora *viz*., *Aspergillus flavus, Aspergillus niger, Cladosporium* sp., *Curvularia* sp. etc. was found with the increase in storage period.

All the fungi that were observed on untreated seeds were also recovered from seeds treated with gamma radiation, but with less per cent seed infection and low levels of abundance (Table 1 and 2). Of the treatments used, gamma radiation at 2.0 kGy (25.56%) was found significantly superior to all other treatments followed by 1.5 kGy (40.41%), 1.0 kGy (57.94%) and the least effective (73.34%) was 0.1 kGy (Table 1). *Alternaria* sp. followed by *Fusarium* sp. and *Aspergillus flavus* were commonly recovered from all the treatments, while *Macrophomina phaseolina, Aspergillus niger* and *Rhizopus* sp. were not recovered from seeds exposed to 2.0 kGy gamma rays. Other fungi *viz., Aspergillus ochraceus, Aspergillus ustus, Emericella nidulans, Epicoccum* sp., *Cladosporium* sp., *Curvularia* sp., *Chaetomium* sp., *Drechslera* sp., *Trichoderma* sp. and *Penicillium* sp. were rarely recovered from the treatments tried and further reduced with the increase in dosage (Table 2). Similar results were reported earlier by Kobori *et al.* (2010) [11], wherein radiation doses of 2-10 kGy inhibited different seed mycoflora of castor in dose dependent manner. Maity *et al.* (2011) [13] also reported that major seedborne fungi were inhibited at a dose of 2.0 kGy and there was gradual decline in seed mycoflora of rice with the increase in gamma radiation from 0.5 to 3.0 kGy. Another study was conducted to know the effect of gamma

radiation (above tested doses) on sunflower seed quality parameters. Results revealed that, with the increase in gamma radiation dosage, germination was reduced. Negative effect on all the seed quality parameters was observed.

Conclusion

Though gamma radiation was effective in reducing the per cent seed infection by different fungi, it affected the germination and other seed quality parameters in negative manner. Effective gamma dosage in terms of reduced per cent seed infection without affecting the seed quality parameters was not found. It concludes that gamma radiated sunflower seed can't be used for seed purpose, So, gamma radiation can't be used as seed treatment in sunflower.

Table 1: Effect of gamma radiation on seed mycoflora of sunflower at different storage periods

Gamma irradiation	Per cent seed infection								
	IAT	1 DAT	1 WAT	2 WAT	3 WAT	1 MAT	2 MAT	3 MAT	Mean
0.1 kGy	70.00* (56.80)**	70.25 (56.96)	71.50 (57.75)	73.75 (59.19)	73.75 (59.19)	75.00 (60.02)	75.75 (60.51)	76.75 (61.19)	73.34
0.3 kGy	70.00 (56.80)	70.00 (56.80)	71.25 (57.58)	71.50 (57.74)	71.75 (57.91)	73.50 (59.03)	73.75 (59.19)	75.00 (60.01)	72.09
0.5 kGy	63.50 (52.84)	65.00 (53.73)	65.00 (53.73)	66.25 (54.49)	66.50 (54.64)	66.75 (54.79)	67.00 (54.95)	67.25 (55.10)	65.91
1.0 kGy	55.00 (47.87)	55.00 (47.87)	56.25 (48.59)	56.75 (48.88)	58.50 (49.89)	60.00 (50.77)	60.75 (51.21)	61.25 (51.50)	57.94
1.5 kGy	38.25 (38.19)	38.25 (38.19)	39.25 (38.78)	40.50 (39.51)	40.50 (39.51)	41.25 (39.95)	42.25 (40.53)	43.00 (40.97)	40.41
2.0 kGy	23.25 (28.80)	23.25 (28.80)	23.50 (28.97)	25.25 (30.14)	25.50 (30.30)	26.25 (30.79)	27.50 (31.60)	30.00 (33.19)	25.56
Control (Carbendazim)	53.25 (46.86)	55.00 (47.87)	56.75 (48.88)	60.00 (50.77)	63.25 (52.69)	70.00 (56.80)	70.00 (56.80)	71.50 (57.73)	62.47
Control (Untreated)	70.00 (56.80)	70.25 (56.96)	73.25 (58.87)	73.75 (59.19)	75.00 (60.02)	75.00 (60.02)	76.75 (61.19)	78.75 (62.58)	74.09
Mean	55.41	55.88	57.09	58.47	59.34	60.97	61.72	62.94	
	Storage period			Gamma irradiation			Storage period x Gamma irradiation		
SE(m)±	0.27			0.27			0.78		
CD at 5%	0.77			0.77			2.19		

IAT - Immediately after treatment
DAT - Day(s) after treatment
WAT - Week(s) after treatment
MAT - Month(s) after treatment
* Mean of four replications
** Figures in parenthesis are angular transformed values

Table 2: Seed mycoflora recovered from sunflower seeds treated with gamma radiation

Gamma irradiation	Alt	Mp	Rhi	Fus	An	Af	Ao	Au	Pen	Tri	En	Epi	Cla	Cha	Cur	Dre
0.1 kGy																
IAT	++	+	+	+	+	+	-	-	-	-	+	-	+	-	-	-
1 DAT	++	+	+	+	+	+	-	-	-	-	+	-	+	-	-	-
1 WAT	++	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-
2 WAT	++	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-
3 WAT	++	-	+	+	+	+	-	-	+	-	-	-	+	-	-	-
1 MAT	+	-	+	+	+	+	-	-	+	-	-	-	+	-	+	-
2 MAT	+	-	+	+	+	+	-	-	+	-	+	+	+	+	+	-
3 MAT	+	-	+	+	+	+	+	+	+	-	-	-	++	+	+	-
0.3 kGy																
IAT	++	+	+	+	+	+	-	-	+	-	+	-	+	+	+	-
1 DAT	++	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-
1 WAT	++	+	+	-	+	+	-	-	-	-	-	-	+	-	-	-
2 WAT	+	-	+	+	+	+	-	-	-	-	-	-	+	-	+	-
3 WAT	+	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-
1 MAT	+	-	+	-	+	+	-	-	+	-	-	-	+	-	-	-
2 MAT	+	-	+	+	+	+	-	+	+	-	-	-	+	+	+	-
3 MAT	+	-	+	+	+	+	+	-	+	-	+	+	+	+	+	-
0.5 kGy																
IAT	++	+	+	-	+	+	-	-	-	-	-	-	+	-	-	-
1 DAT	++	+	+	-	+	+	-	-	-	-	-	-	+	-	-	-
1 WAT	++	+	+	+	+	+	-	-	-	-	+	-	+	-	-	-
2 WAT	++	-	+	+	+	+	-	-	-	-	-	-	+	-	+	-
3 WAT	+	-	+	+	+	+	-	-	-	-	+	-	+	-	-	-
1 MAT	+	-	+	-	+	+	-	-	-	-	-	-	+	-	-	-
2 MAT	+	-	+	+	+	+	-	-	-	-	+	+	+	-	+	-
3 MAT	+	-	+	+	+	+	-	-	-	-	+	+	+	-	+	-
1.0 kGy																

IAT	+	-	+	+	+	+	-	-	+	-	-	-	+	-	+	-
1 DAT	+	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-
1 WAT	+	+	-	-	+	+	-	-	-	-	+	-	-	-	-	-
2 WAT	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-
3 WAT	+	-	+	+	+	+	-	-	-	+	+	-	+	-	-	-
1 MAT	+	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-
2 MAT	+	-	+	-	+	+	-	-	-	-	+	+	+	-	+	+
3 MAT	+	-	+	+	+	+	-	-	+	-	-	+	+	-	+	-
Gamma irradiation	Alt	Mp	Rhi	Fus	An	Af	Ao	Au	Pen	Tri	En	Epi	Cla	Cha	Cur	Dre
1.5 kGy																
IAT	+	+	-	+	+	+	-	-	-	+	-	-	+	-	+	-
1 DAT	+	+	-	+	+	+	-	-	-	-	-	-	+	-	+	-
1 WAT	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
2 WAT	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
3 WAT	+	-	-	+	+	+	-	-	-	-	+	-	+	-	-	-
1 MAT	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
2 MAT	+	-	+	+	+	-	-	-	-	-	+	-	+	-	+	-
3 MAT	+	-	+	+	+	+	-	-	-	-	-	-	+	-	+	-
2.0 kGy																
IAT	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
1 DAT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 WAT	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
2 WAT	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
3 WAT	+	-	-	+	-	-	-	-	-	+	+	-	+	-	+	-
1 MAT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2 MAT	-	-	-	+	-	+	-	-	-		+	-	-	-	+	+
3 MAT	-	-	-	+	-	-	-	-	-	+	-	-	+	-	+	-
Control (Carbendazim)																
IAT	++++	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1 DAT	+++	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1 WAT	+++	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2 WAT	++	+	+	-	+	-	-	-	-	-	+	-	-	-	-	-
3 WAT	++	+	++	-	+	-	-	-	-	-	-	-	+	-	-	-
1 MAT	++	-	++	-	-	-	-	-	-	-	-	-	+	-	-	-
2 MAT	++	-	+++	+	-	-	-	-	-	-	-	-	+	-	+	-
3 MAT	++	-	+++	+	+	+	-	-	-	-	+	-	+	-	+	-
Control (Untreated)																
IAT	+	+	+	+	+	+	-	-	+	-	-	-	+	-	-	-
1 DAT	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-
1 WAT	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
2 WAT	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-
3 WAT	+	+	+	-	+	+	-	-	-	-	-	-	-	-	+	-
1 MAT	+	-	+	-	+	+	-	-	+	+	-	-	+	-	-	-
2 MAT	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	-
3 MAT	+	-	+	-	+	+	+	+	+	-	-	+	+	-	+	+

Alt - *Alternaria* sp., Mp - *Macrophomina phaseolina*, Rhi - *Rhizopus sp.*, Fus - *Fusarium sp.*, An - *Aspergillus niger*, Af - *Aspergillus flavus*, Ao -*Aspergillus ochraceus*, Au - *Aspergillus ustus*, Pen - *Penicillium sp.*, Tri - *Trichoderma sp.*, En - *Emericella nidulans*, Epi - *Epicoccum sp.*, Cla - *Cladosporium sp.*, Cha - *Chaetomium* sp., Cur - *Curvularia sp.*, Dre - *Drechslera* sp. IAT - Immediately after treatment, DAT – Day(s) after treatment, WAT - Week(s) after treatment, MAT - Month(s) after treatment.

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