International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(5): 1363-1367

Received: 14-07-2017
Accepted: 15-08-2017

Srinivas A
Ph.D. Scholar, Department of Plant Pathology, College of Agriculture, PJTSAU, Hyderabad, India

Pushpavathi B
Principal Scientist (Plant Pathology), Seed Research & Technology Centre, PJTSAU, Hyderabad, India

Lakshmi BKM
Scientist (Plant Pathology), Vegetable Research Station, ARI, SKLTSHU, Hyderabad, India

Shashibushan V
Principal Scientist and University Head, Dept. of Entomology, AINP on Pesticide Residues, PJTSAU, Hyderabad, India

Correspondence
Srinivas A
Ph.D. Scholar, Department of Plant Pathology, College of Agriculture, PJTSAU, Hyderabad, India

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

Srinivas A, Pushpavathi B, Lakshmi BKM and Shashibushan V

Abstract
The effect of microwave radiation (1200W, 2450 MHz) for 10, 20, 30, 40, 50 and 60s 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. Among the treatments evaluated, exposing the seeds to microwave radiation for 60s (29.50%) was found significantly superior when compared to other treatments followed by 50s (35.72%), 40s (39.75%) and the least (70.82%) was found with 10s. 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 microwave radiation on sunflower seed quality parameters was also tested and results indicated that microwave radiation can't be used as seed treatment in sunflower.

Keywords: Sunflower seed mycoflora, microwave 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 0.69Mha 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) [15]. 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.

Conventional heat treatments such as heat therapy has been successfully used for the eradication of many bacteria, fungi and viruses in seeds and other planting material (Baker, 1962) [3]. In a search for utilization of novel techniques in agriculture, microwave radiation has been found effective for the control of pests in food processing, crop storage and seed production. It is believed to use heat as the lethal mode of action for controlling pathogens.

Though there were limited evidences reported about effectiveness of microwave radiation in controlling seedborne pathogens benefits such as earlier germination and increased vigour have been reported in previous studies (Tylkowska *et al*., 2010) [17].

In the present study, effect of microwave radiation for different exposure durations 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 ^{0}C. This experiment was conducted at SRTC, Rajendranagar, Hyderabad.

Sunflower seeds were exposed to microwave radiation for 10, 20, 30, 40, 50 and 60s. The microwave radiation treatments were carried out using 1200W LG microwave oven at 2450 MHz radiation. For each treatment the seeds to be exposed to microwave radiation were placed in a 90 mm diameter sterilized glass Petri dish that was placed in the centre of the microwave oven on the rotating plate. After the treatment the seeds were stored in butter paper bags along with chemical (Carbendazim - 0.2%) and untreated control for further use.

The effect of microwave 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 ^{0}C 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) [2] and frequency of the species (Neha and Razia, 2013) [12] 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) [16]. 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) [14], 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 microwave radiation, but with less per cent seed infection and low levels of abundance (Table 1 and 2). Among the treatments evaluated, exposing the seeds to microwave radiation for 60s (29.50%) was found significantly superior when compared to other treatments followed by 50s (35.72%), 40s (39.75%) and the least (70.82%) was found with 10s (Table 1). The fungi *Rhizopus* sp. and *Fusarium* sp. were commonly recovered from all the treatments tested. However, *Alternaria* sp. was found absent in 60s treatment, while *Macrophomina phaseolina* was not found to be associated with seeds exposed to microwave radiation for 40, 50 and 60s. The storage fungus *Aspergillus flavus* followed by *A. niger* were abundant upto 30s exposure of microwave radiation and from 40s to 60s exposure they were drastically reduced and were found only after one month of storage period. Across the exposure durations and storage periods tested, 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 and were further reduced at higher exposure durations (Table 2). In contrast to the present findings, Reddy *et al.* (2000) [13] reported complete elimination of all seed mycoflora of soybean at 30s exposure of microwave radiation. However, with the increase in exposure duration of microwave radiation upto 45s gradual decrease in seed

mycoflora of wheat was also observed by Knox *et al.* (2013) [11].

Another study was conducted to know the effect of microwave radiation (above tested doses) on sunflower seed quality parameters by germination towel method. Results revealed that, with the increase in microwave radiation dosage, germination was reduced. Negative effect on all the seed quality parameters was observed.

Conclusion

Though microwave 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 microwave radiation dosage in terms of reduced per cent seed infection without affecting the seed quality parameters was not found. It concludes that microwave radiated sunflower seed can't be used for seed purpose, So, microwave radiation can't be used as seed treatment in sunflower.

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

Microwave radiation	Per cent seed infection								
	IAT	1 DAT	1 WAT	2 WAT	3 WAT	1 MAT	2 MAT	3 MAT	Mean
10s	68.25* (55.71)**	68.50 (55.87)	70.00 (56.80)	70.00 (56.80)	71.50 (57.74)	73.25 (58.87)	73.50 (59.04)	74.00 (59.36)	70.82
20s	66.50 (54.64)	66.50 (54.64)	67.00 (54.95)	67.50 (55.26)	68.00 (55.56)	68.50 (55.87)	69.50 (56.49)	70.00 (56.80)	67.94
30s	51.25 (45.71)	51.25 (45.71)	51.50 (45.86)	51.50 (45.86)	53.00 (46.72)	53.75 (47.15)	56.75 (48.88)	60.00 (50.77)	53.63
40s	38.25 (38.19)	38.50 (38.34)	38.50 (38.34)	39.50 (38.93)	39.75 (39.07)	40.00 (39.22)	40.50 (39.51)	43.00 (40.97)	39.75
50s	33.25 (35.20)	33.50 (35.35)	35.00 (36.26)	35.00 (36.26)	36.50 (37.15	36.50 (37.15)	37.50 (37.75)	38.50 (38.34)	35.72
60s	26.50 (30.96)	26.75 (31.12)	28.50 (32.24)	30.00 (33.19)	30.00 (33.19)	31.25 (33.97)	31.25 (33.97)	31.75 (34.28)	29.50
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)	73.50 (59.03)	75.00 (60.02)	75.00 (60.02)	75.75 (60.51)	75.75 (60.51)	76.75 (61.19)	74.00
Mean	50.91	51.28	52.59	53.56	54.63	53.68	56.84	58.19	
	Storage period			Microwave radiation			Storage period x Microwave radiation		
SE(m)±	0.27			0.27			0.78		
CD at 5%	0.77			0.77			2.18		

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 microwave radiation

Microwave radiation	Alt	Mp	Rhi	Fus	An	Af	Ao	Au	Pen	Tri	En	Epi	Cla	Cha	Cur	Dre
10s																
IAT	++	+	+	+	++	++	-	-	-	-	-	-	-	-	-	-
1 DAT	++	+	+	+	++	++	-	-	-	-	-	-	-	-	-	-
1 WAT	++	+	+	+	++	++	-	-	-	-	-	-	-	-	-	-
2 WAT	++	+	+	+	++	++	-	-	-	-	-	-	-	-	-	-
3 WAT	++	+	+	+	++	++	-	-	-	-	-	-	-	-	-	-
1 MAT	+	+	+	+	++	++	+	-	-	-	+	-	+	-	-	-
2 MAT	+	-	+	+	++	++	-	-	+	-	+	-	+	+	-	-
3 MAT	+	-	+	+	++	++	+	-	+	-	+	+	+	-	-	+
20s																
IAT	++	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
1 DAT	++	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
1 WAT	++	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
2 WAT	++	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
3 WAT	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
1 MAT	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	-
2 MAT	+	-	+	+	+	++	-	-	+	-	-	-	+	-	-	-
3 MAT	+	-	+	+	+	++	+	-	+	-	+	-	+	+	+	-
30s																
IAT	+	+	+	-	+	+	-	-	+	-	-	-	-	-	-	-
1 DAT	+	+	+	-	+	+	-	-	+	-	-	-	-	-	-	-
1 WAT	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
2 WAT	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
3 WAT	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
1 MAT	+	-	+	+	+	+	+	+	-	-	+	-	-	-	-	-

2 MAT	+	-	++	+	+	+	-	-	+	-	-	-	+	-	-	-
3 MAT	+	-	++	+	+	+	+	-	+	+	+	-	+	-	-	-
40s																
IAT	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-
1 DAT	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1 WAT	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2 WAT	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
3 WAT	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
1 MAT	+	-	+	+	+	+	-	-	-	-	+	-	-	-	-	-
2 MAT	+	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-
3 MAT	-	-	+	+	+	+	-	-	+	+	+	-	-	+	+	-
Microwave radiation	**Alt**	**Mp**	**Rhi**	**Fus**	**An**	**Af**	**Ao**	**Au**	**Pen**	**Tri**	**En**	**Epi**	**Cla**	**Cha**	**Cur**	**Dre**
50s																
IAT	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-
1 DAT	+	-	+		-	-	-	-	-	-	-	-	-	-	-	-
1 WAT	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2 WAT	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3 WAT	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1 MAT	+	-	+	+	+	+	-	-	+	+	+	-	-	-	-	-
2 MAT	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-
3 MAT	-	-	+	+	-	+	-	-	-	+	+	-	-	-	-	-
60s																
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.

References

1. Afzal R, Mughal SM, Munir M, Sultana K, Qureshi R, Arshad M, *et al.* Laghari MK. Mycoflora associated with seeds of different sunflower cultivars and its management. Pakistan Journal of Botany. 2010; 42(1):435-445.
2. Aslam MF, Irshad G, Naz F, Aslam MN, Ahmed R. Effect of seed-borne mycoflora on germination and fatty acid profile of peanuts. Pakistan Journal of Phytopathology. 2015; 27(2):131-138.
3. Baker KF. Thermotherapy of planting material. *Phytopathology*. 1962; 52:1244-1255.
4. Barnett HL. Illustrated Genera of Imperfect Fungi 2nd Edition, Burgess Publishing Company, USA, 1965; 1-220.
5. Booth C. The Genus Fusarium. Commonwealth Mycological Institute, Eastern Press Limited, Kew, England, 1971; 1-40.
6. Commonwealth Mycological Institute CMI. Description of Pathogenic Fungi and Bacteria. Kew, England, 1970; 1-100.
7. Gomez KA, Gomez AA. Statistical Procedures for Agricultural Research 2nd Edition, John Willey and Sons, New York, 1984; 316-356.
8. Indiastat. http://www.indiastat.com/agriculture/2/ commercialcrops/17188/ oilseeds/17204/stats.aspx, 2013-14.

9. ISTA International Seed Testing Association. International rules for seed testing. Seed Science and Technology, 1996; 24:1-335.
10. Jamaria SL, Sharma KP, Gupta RBL. Fungi intercepted from sunflower seeds and their control. Indian Journal of Plant Pathology, 1975; 5:212-213.
11. Knox OGG, Mchugha MJ, Fountaine JM, Havisa ND. Effects of microwaves on fungal pathogens of wheat seed. Crop Protection, 2013; 50:12-16.
12. Neha P, Razia KZ. Comparative study of seed dressing fungicides and Calotropis procera latex for the control of seedborne mycoflora of wheat. Annals of Biological Research. 2013; 4(4):1-6.
13. Reddy P, Mycock DJ, Berjak P. The effect of microwave irradiation on the ultra structure and internal fungi of soybean seed tissues. Seed Science and Technology. 2000; 28(2):277-289.
14. Subramanian CV. Hyphomycetes. Indian Council of Agricultural Research, New Delhi, 1971; 1-250.
15. Tanaka B, Maeda JA, Plazas I. Fungal microflora con seeds storage environment. Scientia Agricola, 2001; 58:501-508.
16. Tuite J. Plant Pathological Methods, Fungi and Bacteria. Burgess Publishing company, USA, 1969; 229.
17. Tylkowska K, Turek M, Prieto RB. Health, germination and vigour of common bean seeds in relation to microwave irradiation. *Phytopathologia*, 2010; 55:5-12.