www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(9): 256-259

www.thepharmajournal.com
Received: 17-07-2017
Accepted: 18-08-2017

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

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

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

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

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

Detection of Seedborne Mycoflora of Sunflower (*Helianthus annuus* L.)

A Srinivas, B Pushpavathi, BKM Lakshmi and V Shashibhushan

Abstract
The sunflower seeds were subjected to standard blotter and agar plate methods as recommended by ISTA, for detection of seedborne mycoflora. Seventeen fungi belonging to fourteen genera were detected through standard blotter method, whereas thirteen fungi belonging to eleven genera were recorded through agar plate method. Several pathogenic and storage fungi were observed in both the methods. Among the fungi detected, frequency of *Alternaria* sp. was found significantly highest in both the methods (standard blotter method - 27.00% and agar plate method - 20.00%). Of the two methods tested for detection of seed mycoflora, standard blotter method was found superior over agar plate method.

Keywords: Sunflower seed mycoflora, Storage mycoflora, Frequency, Standard blotter method, Agar plate 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 and important nutrients, selenium, copper, zinc, vitamin E and B complex as well (Afzal *et al*., 2010) [1]. It is a rich source of linoleic acid (64%) which helps in reducing the cholesterol deposition. The total area of sunflower in India is 0.69Mha with a production of 0.50Mt. It occupies 6th place among the oilseed crops grown in India in terms of production (Indiastat, 2013-14) [9]. 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) [18]. 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*). In addition to these seedborne pathogens, seeds are also known to harbour several other fungi which may cause seed rot, seedling mortality, reduced seedling vigour and seed viability which leads to poor plant stand in the field. The seed quality also affects the rate and uniformity of emergence and the dynamics of initial plant growth. The seedborne fungi may also cause systemic or local infections, resulting in development of diseases at later stages of the crop growth. It was reported that, 20-30 per cent loss in germinability of sunflower was due to seedborne diseases (Jamaria *et al.*, 1975) [11]. Therefore, management of seedborne fungi is extremely important for realization of full yield potential of cultivars. In the present study, detection of different seed mycoflora of local popular cultivar of sunflower was taken up.

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.

Detection of seed mycoflora
To detect the seed mycoflora associated with sunflower seeds, two standard detection methods, *viz*., standard blotter method and agar plate method (ISTA, 1996) [10] were employed**.** The experiment was conducted with four replications and under each replication hundred seeds were tested. The seeds were placed in 90mm Petri plates @ 10 seeds per plate and 10 such plates were maintained under each replication.

The mycoflora observed on seeds were isolated and identified.

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 and the plates were incubated at 24 ± 2 ^{0}C for seven days in an incubator.

Agar plate method
PDA was prepared and 20ml of the medium was distributed into each of the sterile Petri plates under aseptic conditions. Seeds were transferred to the plates containing solidified PDA medium. Ten seeds per plate were placed at equidistance on medium and the plates were incubated at 24 ± 2 °C in an incubator for seven days.

Data recording
On 8th day, the seeds incubated under both the detection methods 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) [14] the following formulae were used.

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

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

To determine the quantitative differences between the fungi colonized on seeds, the relative abundance of an individual species was calculated as follows (Neha and Razia, 2013) [14].

$$\text{Relative abundance} = \frac{\text{Total no. of colonies of an individual fungus}}{\text{Total number of colonies of all fungi}} \times 100$$

The data obtained was statistically analyzed using factorial CRD.

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) [19]. Pure cultures thus obtained were maintained on PDA slants.

Identification of Fungi
Identification of various seed mycoflora obtained by the above two methods was done using relevant keys given by Subramanian (1971) [17], Booth (1971) [4], Barnett and Hunter (2003) [3] and descriptions of CMI (1970) [5].

Results and Discussion
Standard blotter method
The data pertaining to estimation of seed mycoflora of sunflower analysed through standard blotter method is presented in Table 1 and 2. Results indicated that, 17 fungi belonging to 14 genera *viz*., *Alternaria* sp., *Macrophomina phaseolina, Fusarium* sp., *Drechslera* sp., *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Aspergillus ustus, Emericella nidulans, Rhizopus* sp., *Mucor* sp., *Epicoccum* sp., *Cladosporium* sp., *Curvularia* sp., *Chaetomium* sp., *Trichoderma* sp. and *Penicillium* sp. were observed on sunflower seeds. The mean per cent seed infection due to collective colonization by all these fungi was recorded as 66.75% (Table 1). However, among the fungi observed, the frequency of occurrence of *Alternaria* sp. was significantly highest (27.00%) followed by *Aspergillus niger* (17.00%), *Aspergillus flavus* (13.00%) and the least was found with *Drechslera* sp. (3.00%) which was at par with *Aspergillus ochraceus* (4.00%), *Aspergillus ustus* (4.00%), *Emericella nidulans* (5.00%), *Epicoccum* sp. (5.00%), *Chaetomium* sp. (4.00%) and *Trichoderma* sp. (5.00%). Similar trend was observed for relative abundance, wherein it was highest for *Alternaria* sp. (19.15%) followed by *Aspergillus niger* (12.06%), *Aspergillus flavus* (9.22%) and the least of that was observed in case of *Drechslera* sp. (2.13%). The dominance of *Alternaria* sp. on sunflower seeds was also reported earlier by Raj *et al*. (2007) [15] and Ghoneem *et al*. (2014) [8].

Agar plate method
The sunflower seeds were also analysed by agar plate method for detection of mycoflora associated with the seeds. Results indicated that, 13 fungi belonging to 11 genera *viz*., *Alternaria sp., Macrophomina phaseolina, Fusarium* sp., *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Rhizopus* sp., *Mucor* sp., *Epicoccum* sp., *Cladosporium* sp., *Curvularia* sp., *Trichoderma* sp. and *Penicillium* sp. were recorded through agar plate method on sunflower seeds with a mean per cent seed infection of 60.25% (Table 1). Among these fungi, the significantly highest frequency was recorded with *Alternaria* sp. (20.00%) which was at par with *Rhizopus* sp. (18.00%) followed by *Macrophomina phaseolina* (13.00%) and the least (2.00%) was found with *Aspergillus ochraceus* (Table 2). Similar trend was observed for relative abundance, wherein it was highest for *Alternaria* sp. (17.54%) which was at par with *Rhizopus* sp. (15.79%) followed by *Macrophomina phaseolina* (11.40%) and the least was found with *Aspergillus ochraceus* (1.75%). In support of these results, the highest frequency of *Alternaria* sp. on sunflower seeds by agar plate method was also reported earlier by Levic *et al*. (2012) [12], El-Wakil (2014) [7] and Mardare *et al.* (2014) [13].

Comparison between standard blotter and agar plate methods
A total of 17 fungi were recorded through standard blotter method, whereas 13 fungi were recorded by agar plate method. The fungi that were observed only through standard blotter method include *Drechslera* sp., *Aspergillus ustus*, *Emericella nidulans* and *Chaetomium* sp. The mean per cent seed infection recorded was significantly high in standard blotter method (66.75%) than that in agar plate method

(60.25%). The mean frequency of fungi recorded was also found to be significantly highest in standard blotter method (8.29%) when compared to agar plate method (6.71%). The fungi *viz.*, *Macrophomina phaseolina* (13.00%) and *Rhizopus* sp. (18.00%) were recorded with significantly high frequency by agar plate method when compared to standard blotter method. The standard blotter method was found to be superior in recovering most of the other fungi associated with sunflower seeds. The superiority of standard blotter method over agar plate method was also reported earlier by Dawar and Ghaffar (1991) [6] and Salustiano *et al.* (2006) [16] in sunflower.

Table 1: Mean per cent seed infection by seed mycoflora of sunflower.

S. No.	Detection method	Per cent seed infection
1	Standard blotter method	66.75
2	Agar plate method	60.25

Table 2: Frequency and relative abundance of seed mycoflora associated with sunflower seeds

S. No.	Name of the fungus	Frequency			Relative abundance		
		SBM	APM	Mean	SBM	APM	Mean
1	*Alternaria* sp.	27.00***** (31.23)**	20.00 (26.48)	23.50	19.15 (26.06)	17.54 (24.80)	18.35
2	*Macrophomina phaseolina*	8.00 (16.13)	13.00 (21.03)	10.50	5.67 (13.46)	11.40 (19.69)	8.54
3	*Fusarium* sp.	7.00 (14.96)	7.00 (15.24)	7.00	4.96 (12.75)	6.14 (14.27)	5.55
4	*Drechslera* sp.	3.00 (9.78)	0.00 (0.00)	1.50	2.13 (8.17)	0.00 (0.00)	1.06
5	*Aspergillus flavus*	13.00 (21.03)	10.00 (18.37)	11.50	9.22 (17.57)	8.77 (17.25)	9.00
6	*Aspergillus niger*	17.00 (24.22)	12.00 (20.17)	14.50	12.06 (20.18)	10.53 (18.92)	11.29
7	*Aspergillus ochraceus*	4.00 (11.34)	2.00 (7.99)	3.00	2.84 (9.48)	1.75 (7.49)	2.30
8	*Aspergillus ustus*	4.00 (11.34)	0.00 (0.00)	2.00	2.84 (9.54)	0.00 (0.00)	1.42
9	*Emericella nidulans*	5.00 (12.75)	0.00 (0.00)	2.50	3.55 (10.69)	0.00 (0.00)	1.77
10	*Rhizopus* sp.	10.00 (18.35)	18.00 (25.05)	14.00	7.09 (15.36)	15.79 (23.51)	11.44
11	*Mucor* sp.	4.00 (11.34)	3.00 (9.78)	3.50	2.84 (9.54)	2.63 (9.16)	2.73
12	*Epicoccum* sp.	5.00 (12.82)	5.00 (12.82)	5.00	3.55 (10.85)	4.39 (12.02)	3.97
13	*Cladosporium* sp.	7.00 (15.24)	5.00 (12.82)	6.00	4.96 (12.76)	4.39 (12.12)	4.68
14	*Curvularia* sp.	10.00 (18.37)	7.00 (15.21)	8.50	7.09 (15.39)	6.14 (14.22)	6.62
15	*Chaetomium* sp.	4.00 (11.48)	0.00 (0.00)	2.00	2.84 (9.71)	0.00 (0.00)	1.42
16	*Trichoderma* sp.	5.00 (12.82)	5.00 (12.71)	5.00	3.55 (10.75)	4.39 (11.88)	3.97
17	*Penicillium* sp.	8.00 (16.34)	7.00 (15.21)	7.50	5.67 (13.71)	6.14 (14.22)	5.91
	Mean	8.29	6.71		5.88	5.88	
		Fungi	Methods	Fungi x Methods	Fungi	Methods	Fungi x Methods
	SE(m)±	0.79	0.27	1.12	0.53	0.18	0.75
	CD at 5%	2.22	0.76	3.14	1.50	0.51	2.12

References

1. Afzal R, Mughal SM, Munir M, Sultana K, Qureshi R, Arshad M *et al*. 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. Barnett HL, Hunter DB. Illustrated Genera of Imperfect Fungi. Burgess Publishing Company, USA, 1972.
4. Booth C. The Genus Fusarium. Commonwealth Mycological Institute, Eastern Press Limited, Kew, 1971.
5. Common wealth Mycological Institute (CMI). Description of pathogenic fungi and bacteria. Kew, England, 1970.
6. Dawar S, Ghaffar A. Location of fungi in sunflower seed. Pakistan Journal of Botany. 1990; 22(2):117-120.
7. El-Wakil DA. Seed-borne fungi of sunflower (*Helianthus annuus* L.) and their impact on oil quality. IOSR Journal of Agriculture and Veterinary Science. 2014; 6(6):38-44.
8. Ghoneem KM, Ezzat SM, El-Dadamony NM. Seedborne fungi of sunflower in Egypt with reference to pathogenic effects and their transmission. *Plant Pathology Journal*. 2014; 13(4):278-284.
9. Indiastat. 2013-14. http://www.indiastat.com/agriculture/2/commercialcrops/17188/oilse eds/17204/stats.aspx.
10. ISTA. (International Seed Testing Association), International rules for seed testing. Seed Science and Technology. 1996; 24:1-335.
11. Jamaria SL, Sharma KP, Gupta RBL. Fungi intercepted from sunflower seeds and their control. Indian Journal of Plant Pathology. 1975; 5:212-213.
12. Levic J, Stankovic S, Krnjaja V, Stancic AB, Ivanovic D. Distribution frequency and incidence of seed-borne pathogens of some cereals and industrial crops in serbia. *Pesticidii fitomedicina*. 2012; 27(1):33-40.
13. Mardare ES, Cristea S, Zala RC. Researches on the micoflora of sunflower's achenes for the hybrids cultivated in fetesti area, Ialomita county. *Seria Agronomie*. 2014; 57(2):213-216.
14. 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.
15. Raj MH, Niranjana SR, Nayaka SC, Shetty HS. Health status of farmers saved paddy, sorghum, sunflower and cow pea seeds in Karnataka, India. World Journal of Agricultural Sciences. 2007; 3(2):167-177.
16. Salustiano ME, Machado JDC, Pittis JE. Comparison of two methods in the health detection of *Alternaria helianthi* in sunflower seeds. *Phytopathology Brazilian*. 2006; 31(3):322-324.
17. Subramanian CV. *Hyphomycetes*. Indian Council of Agricultural Research, New Delhi, 1971.

18. Tanaka B, Maeda JA, Plazas I. Fungal microflora con seeds storage environment. *Scientia Agricola*. 2001; 58:501-508.
19. Tuite J. Plant Pathological Methods, Fungi and Bacteria. Burgess Publishing company, USA. 1969, 229.