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SEED-BORNE MYCOFLORA OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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Abstract

Using standard blotter and deep-freezing techniques, seed-borne mycoflora of 35 samples of sunflower (*Helianthus annuus* L.) were studied. *Acremonium fusidioides, Arthrobotrys oligospora, Aspergillus ochraceus, Bipolaris bisepta, Cephaliophora tropica, Chaetomium spinosum, Cladobotryum varium, Cladosporium cladosporioides, Emericella nidulans, Gonatobotrys simplex, Humicola grisea, Memnoniella echinata, Mucor mucedo, Myrothecium verrucaria, Phialophora verrucosa* and *Syncephalastrum racemosum* were found to be new seed-borne fungal species on sunflower. *Absidia corymbifera, Alternaria alternata, Aspergillus flavus, A. niger, A. terreus, Chaetomium bostrychodes, C. globosum, Emericella nidulans, Fusarium pallidoroseum, F. solani, Macrophomina phaseolina, Penicillium* spp., *Rhizoctonia solani* and *Rhizopus stolonifer* were predominantly isolated by both techniques. During seed component plating, *Aspergillus awamori, A. ustus* and *Exerohilum halodes* were found to be new reported species. *Macrophomina phaseolina, Rhizoctonia solani* and *Trichoderma harzianum* were isolated from all component parts, whereas, *Fusarium solani* was isolated only from cotyledons and axis.

Introduction

Sunflower (*Helianthus annuus* L.) is an annual ornamental herb grown as an oil seed crop. It is planted in Pakistan over an area of 61,900 hectares producing 87,100 tons annually (Anon., 2002). Seeds, which are consumed as raw, roasted or salted, contain 32 to 45% edible oil, which is a rich source of polyunsaturated fatty acid. Several seed-borne fungi including species of *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Drechslera*, *Fusarium* and *Penicillium* have been reported from sunflower seeds (Reddy, 1989; Kaur *et al*., 1990; Shahnaz & Ghaffar, 1991). Moreover, seed-borne fungi decrease protein, carbohydrate, cholesterol contents, iodine values and increase acid quantity (Singh & Prasad, 1986; Sexana & Karan, 1991; Ahmad *et al*., 1994). Low quality with reduced and discolored oil contents of sunflower seeds are reported to be caused by species of *Rhizopus* (Zad, 1979; Singh & Prasad, 1977), whereas seed infection and biodeterioration during storage and reduction in germination is reported to be caused by *Alternaria alternata* (Prasad & Singh, 1983). However, leaf blight, floral blight and fruit infection are also reported on sunflower (Kumar *et al.*, 1997; Svetov, 1975; Kumar & Dwivedi, 1981). Association of *Fusarium* species with seeds results in spread of several diseases in fields such as wilting (Vijayalakshmi & Rao, 1986) foot rot, seedling blight, stunting, wilting and hypertrophy in sunflower (Shahnaz & Ghaffar, 1990, 1991a). Straser (1985) reported *Fusarium oxysporum* as seed borne pathogen of sunflower even from the endosperm of chemically treated seeds. In the present study fungi associated with sunflower seeds were detected by standard blotter, deep-freezing and seed component plating techniques. The mycoflora was compared with that reported by Iftikhar *et al*., (1993), Richardson (1979, 1981, 1983) and Abbas *et al*., (2004).

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Materials and Methods

Thirty-five seed samples of *Helianthus annuus* L. (sunflower) were collected from different localities of Karachi, Sindh. These samples were analyzed for the presence of seed-borne mycoflora by standard blotter (Anon., 1976) and deep-freezing techniques (Limonard, 1968). Four hundred seeds of each sample were plated on 3 layered moistened blotter discs in 9 cm glass Petri plates @ 15 seeds per plate and incubated for 7 days at 22±1°C in Eyela La 1000 low temperature incubator. Incubated seeds were examined under compound light microscope at 4 - 40X magnifications. In deep-freezing technique, seeds were incubated at 22±1°C for 24 hrs., followed by an incubation period at -20±1°C for 24 hrs., and then at 22±1°C for 5 days.

Six selected samples of sunflower seeds (that showed highest occurrence of pathogenic fungi, during seed testing techniques) were further tested to detect location of seed-borne fungi in various parts of sunflower seeds using seed component plating technique (Mathur *et al.,* 1975). Twenty-five seeds of each sample were soaked for 10 hrs. in 10 ml of sterilized distilled water in test tubes and dissected aseptically into seed coat (testa and tegmen), cotyledons and embryo (Willis, 1960). Component parts were treated with 5% sodium hypochlorite and plated on PDA. In all methods fungi were isolated and purified on potato dextrose agar (PDA), corn meal agar (CMA) and speziellier nahrstoffarmer agar (SNA). The isolated fungi were identified after reference to Booth (1971), Ellis (1971), Barnett & Hunter (1972), Carmichael *et al*., (1980), Domsch *et al*., (1980), Nelson *et al*., (1983), Joffe (1986), Pascoe (1990 a, b), Nirenberg (1990) and Singh *et al*., (1991). The data was statistically analyzed using computer-based software SPSS version 10.

Results and Discussion

Using standard blotter technique, 45 fungal species belonging to 27 genera and by deep-freezing technique, 38 fungal species belonging to 23 genera were isolated and identified from 35 samples of *Helianthus annuus* (Table 1). Occurrence of fungi was recorded in terms of mean value with standard error and standard deviation. *Acremonium fusidioides*, *Arthrobotrys oligospora*, *Aspergillus ochraceus*, *Bipolaris bisepta*, *Cephaliophora tropica*, *Chaetomium spinosum*, *Cladobotryum varium*, *Cladosporium cladosporioides*, *Emericella nidulans*, *Gonatobotrys simplex*, *Humicola grisea*, *Memnoniella echinata*, *Mucor mucedo*, *Myrothecium verrucaria*, *Phialophora verrucosa* and *Syncephalastrum racemosum* were found to be new records of seed-borne fungal species on sunflower.

A comparision of two techniques showed that *Absidia corymbifera*, *Acremonium fusidioides*, *Alternaria alternata*, *Aspergillus candidus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. sulphureus*, *A. tamarii*, *A. tereus*, *A. versicolor*, *Bipolaris hawaiiensis*, *Cephalosporium* sp., *Chaetomium bostrychodes*, *C. globosum*, *Curvularia lunata*, *Emericella nidulans*, *Exerohilum rostratum*, *Fusarium chlamydosporum*, *F. pallidoroseum*, *F. solani*, *Macrophomina phaseolina*, *Penicillium* spp., *Phoma oleracea*, *Rhizoctonia solani*, *Rhizopus stolonifer*, and *Syncephalastrum racemosum* were commonly isolated by both techniques. On the other hand, *Arthrobotrys oligospora*, *Cephaliophora irregularis*, *C. tropica*, *Chaetomium crispatum*, *C. spinosum*, *Cladobotryum varium*, *Cladosporium cladosporioides*, *Curvularia pallescens*, *Cylindrocarpon* sp., *Emericella nidulans*, *Fusarium equiseti*, *F. proliferatum*,

Gonatobotrys simplex, *Memnoniella ehinata*, *Myrothecium verrucaria* and *Verticillium* sp. were isolated only by standard blotter technique, and *Bipolaris australiensis*, *B. bisepta*, *Fusarium oxysporum*, *F. sporotrichioides*, *Humicola grisea*, *Mucor mucedo*, *Phialophora verrucosa*, *Scopulariopsis* sp. *Stachybotrys atra* and *Ulocladium* sp., by only deep-freezing technique (Table 1).

Deep freezing technique appeared more suitable as compared to standard blotter technique for the detection of *Fusarium* spp. In the present study, 8 *Fusarium* spp., were isolated from sunflower seed samples, where *F. pallidoroseum* and *F. solani* were found predominantely and commonly isolated by both techniques as compared to the reports of Shahnaz & Ghaffar (1991a) where 5 *Fusarium* spp., were reported with predominant occurrence of *F. moniliforme* and *F. solani*. *Fusarium oxysporum* and *F. solani* which were isolated from seeds are aggressive pathogens of sunflower as compared to *F. moniliforme* and *F. pallidoroseum* (Bhutta *et al.*, 1997).

Apart from *Fusarium* spp. some other pathogenic fungi such as *Alternaria alternata*, *Curvularia lunata*, *Macrophomina phaseolina*, *Myrothecium roridum*, *Phoma oleracea*, *Rhizoctonia solani* and *Verticillium dahliae* were also isolated from sunflower seeds. *Myrothecium verrucaria* and *Phoma oleracea* were isolated for the first time from sunflower seeds. It may be mentioned that *Rhizoctonia solani* which is an important pathogen of sunflower (Ahmad *et al*., 1994) was also isolated from various parts of seeds during component plating, indicating its systemic nature.

Nine species of *Aspergillus* were isolated from seed samples and all of them are reported to produce different groups of aflatoxins which are natural toxins and hazardous to animals and man (Shahnaz & Ghaffar, 1991a,b; Abdel-Mallek *et al*., 1994). Among them, *Aspergillus flavus* and *A. niger* showed highest occurrence, that may lower the seed quality. Various threatening diseases including different types of carcinoma in humans may develop, if such seeds are consumed as food.

During seed component plating of selected samples, a total of 24 fungal species belonging to 17 genera were isolated and identified, mostly from testa and cotyledons as compared to tegmen and embryo. *Aspergillus awamori*, *A. ustus* and *Exerohilum halodes* were found to be new reported species. Intra- and extra-embryal seed-borne pathogenic fungi *viz*., *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium solani* were predominantely isolated from all components of seeds, corroborating the findings of Shahnaz & Ghaffar (1990), but contrary to the report of Sadashivaiah (1986) who found *Macrophomina phaseolina* infection only in testa and tegmen. *Macrophomina phaseolina* and *Rhizoctonia soloni* were more predominantely detected from seed coat and cotyledons (extra embryl), whereas infection of *Fusarium solani* was observed in embryo and cotyledons (intra embryl). These pathogens may colonize the growing roots and cause rotting of germinating seeds. All tested samples showed 100% colonization of *Aspergillus flavus, A. niger and Rhizopus stolonifer*. A number of other saprophytic fungi were identified as *Absidia corymbifera*, *Aspergillus awamori, A. fumigatus, A. ochraceus, A. terreus, A. ustus, A. versicolor*, *Bipolaris australiensis*, *B. hawaiiensis*, *Chaetomium* sp., *Curvularia lunata*, *Emericella nidulans*, *Exerohilum halodes*, *Paecillomyces variotii*, *Penicillium* sp., *Rhizopus stolonifer*, *Syncephalastrum racemosum* and *Trichoderma harzianum*. The study of mycoflora by component plating technique appeared helpful in detecting the deep seated seed infection. Pericarp and seed coat may be removed or cleaned properly if infection is superficial. However, in case of deep-seated fungal infection especially those producing mycotoxins, seed lots must be rejected and destroyed. This technique of ISTA is also helpful in selecting healthy seed lots for raising new plants.

Table 1. Seed-borne mycoflora of *Helianthus annuus* (All values are in percentages).

No.	Mycoflora	Blotter technique			Deep freezing technique		
		Occ.	Mean ± SE	Range	Occ.	Mean ± SE	Range
1.	*Absidia corymbifera*	11.42	0.31 ± 0.25	0.25-8.75	65.71	3.15 ± 0.90	0.25-17.5
2.	*Acremonium fusidioides*	5.71	2.56 ± 1.43	0.25-0.5	31.42	1.14 ± 0.33	0.25-16.75
3.	*Alternaira alternata*	51.42	2.85 ± 0.88	0.25-16.75	74.28	3.83 ± 0.79	0.25-16.75
4.	*Arthrobotrys oligospora*	2.85	0.007 ± 0.007	0.25	—	—	—
5.	*Aspergillus candidus*	11.42	0.57 ± 0.48	0.25-1.5	17.14	1.14 ± 0.91	0.5-2.5
6.	*A. flavus*	82.85	8.32 ± 1.64	0.75-41.0	60	3.38 ± 0.72	0.75-16.75
7.	*A. fumigatus*	11.42	2.03 ± 0.94	0.25-2.5	8.57	0.12 ± 0.08	0.5-2.5
8.	*A. niger*	85.71	6.39 ± 1.13	0.5-24.25	54.28	2.96 ± 0.64	0.25-12
9.	*A. ochraceus*	14.28	0.11 ± 0.05	0.25-1.25	11.42	0.07 ± 0.03	0.25-1.0
10.	*A. sulphureus*	20	1.64 ± 0.92	0.5-4.0	14.28	0.32 ± 0.14	1.5-4.0
11.	*A. tamarii*	25.7	0.44 ± 0.21	0.25-6.0	20	0.48 ± 0.20	0.25-5.0
12.	*A. terreus*	68.57	1.83 ± 0.44	0.25-10.5	45.71	1.00 ± 0.30	0.25-8.0
13.	*A. versicolor*	37.14	0.44 ± 0.18	0.25-5.25	22.85	0.94 ± 0.74	0.25-1.0
14.	*Bipolaris australiensis*	—	—	—	11.42	0.17 ± 0.09	0.5-2.5
15.	*B. bisepta*	—	—	—	5.71	0.03 ± 0.02	0.25-0.75
16.	*B. hawaiiensis*	14.28	0.10 ± 0.04	0.25-1.25	20	0.80 ± 0.34	1.5-10.5
17.	*Cephaliophora irregularis*	5.71	0.01 ± 0.009	0.25	—	—	—
18.	*C. tropica*	2.85	0.05 ± 0.05	2.0	—	—	—
19.	*Cephalosporium sp.*	17.14	0.25 ± 0.14	0.5-5.0	11.42	0.05 ± 0.02	0.25-0.75
20.	*Chaetomium bostrychodes*	28.57	0.59 ± 0.23	0.5-7.25	37.14	1.58 ± 0.45	0.5-8.25
21.	*C. crispatum*	5.71	0.05 ± 0.05	0.25-1.75	—	—	—
22.	*C. globossum*	51.42	1.52 ± 0.38	0.25-5.25	22.85	3.01 ± 1.14	1.0-30.5
23.	*C. spinosum*	11.42	0.13 ± 0.07	0.25-2.0	—	—	—
24.	*Cladobotrym varium*	8.57	0.87 ± 0.85	0.25	—	—	—
25.	*Cladosporium cladosporioides*	2.85	0.01 ± 0.01	0.5	—	—	—
26.	*Curvularia lunata*	20	0.17 ± 0.09	0.25-1.25	20	0.43 ± 0.17	0.25-4.5
27.	*C. pallescens*	5.71	0.05 ± 0.03	0.5-1.25	—	—	—
28.	*Cylindrocarpon* sp.	5.71	0.01 ± 0.009	0.25	—	—	—

Table 1 (Cont'd.)

No.	Mycoflora	Blotter technique			Deep freezing technique		
		Occ.	Mean ± SE	Range	Occ.	Mean ± SE	Range
29.	*Emericella nidulans*	5.71	0.47 ± 0.37	0.5-3.0	—	—	—
30.	*Emericella* sp.	42.85	0.61 ± 0.33	0.25-11.5	48.57	7.82 ± 1.98	1.5-40.0
31.	*Exerohilum rostratum*	5.71	0.01 ± 0.009	0.25	28.57	0.60 ± 0.19	0.5-4.0
32.	*Fusarium chlamydosporum*	2.85	0.007 ± 0.007	0.25	5.71	0.03 ± 0.02	0.25-0.75
33.	*F. equiseti*	5.71	0.02 ± 0.01	0.25-0.5	—	—	—
34.	*F. moniliforme*	17.14	0.64 ± 0.54	0.2-0.25	—	—	—
35.	*F. oxysporum*	—	—	—	5.71	0.03 ± 0.02	0.25-0.75
36.	*F. pallidoroseum*	14.28	0.04 ± 0.19	0.25-0.5	22.85	0.59 ± 0.22	0.5-5.5
37.	*F. proliferatum*	8.57	0.04 ± 0.02	0.25-0.75	—	—	—
38.	*F. solani*	20	0.09 ± 0.03	0.25-1.0	28.57	0.61 ± 0.20	0.5-4.5
39.	*F. sporotrichioides*	—	—	—	14.42	0.33 ± 0.22	0.5-2.75
40.	*Gonatobotrys simplex*	5.71	0.55±0.46	0.25-3.5	—	—	—
41.	*Humicola grisea*	—	—	—	14.28	0.05 ± 0.02	0.25-0.75
42.	*Macrophomina phaseolina*	37.14	0.44 ± 0.18	0.25-4.0	37.14	0.44 ± 0.18	0.25-2.0
43.	*Memnoniella echinata*	2.85	0.007 ± 0.007	0.25	—	—	—
44.	*Mucor mucedo*	—	—	—	22.85	0.53 ± 0.24	0.25-8.0
45.	*Myrothecium verrucaria*	5.71	0.04 ± 0.03	0.5-1.0	—	—	—
46.	*Penicillium* spp.	34.2	1.29 ± 0.69	0.25-18.25	42.85	13.23 ± 3.42	2.5-70.5
47.	*Phialophora verrucosa*	—	—	—	34.28	3.60 ± 0.96	8.25-15.25
48.	*Phoma oleracea*	8.57	0.02 ± 0.01	0.25	17.14	2.36 ± 1.01	2.75-16.5
49.	*Rhizoctonia solani*	14.28	0.07 ± 0.03	0.25-1.25	11.42	0.16 ± 0.09	0.5-2.5
50.	*Rhizopus stolonifer*	71.42	6.60 ± 1.50	1.5-37.75	42.85	21.66 ± 4.94	8.25-90.0
51.	*Scopulariopsis* sp.	—	—	—	42.85	0.29 ± 0.10	0.25-2.5
52.	*Stachybotrys atra*	—	—	—	14.28	0.10 ± 0.04	0.25-1.25
53.	*Syncephalastrum racemosum*	8.57	0.22 ± 0.17	0.5-6.0	8.57	0.20 ± 0.17	0.25-6.0
54.	*Verticillium* sp.	2.85	0.52 ± 0.45	2.25	—	—	—
55.	*Ulocladium* sp.	—	—	—	22.85	0.69 ± 0.25	1.25-6.25

Damages of seeds, such as seed death, seedling and plant abnormalities or decreased seed vigor caused by seed-borne pathogens are not always recognized by users. Once harmful fungi, pathogenic as well as toxigenic, have been listed, it is important to define for each of them the methods to be used for their detection and identification (Neergaard, 1979). When basic knowledge of the fungus and mycotoxin(s) is obtained, progress in the prevention and control could be rapid. There is undoubtedly worldwide contamination of the seeds with a variety of mycotoxin producing fungi and there is little doubt that mycotoxins are a probable source of naturally occurring carcinogens in humans (Diener *et al*., 1981). Concerted effort could be made to avoid such contaminants using seed health technology.

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