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DETECTION OF THE SEEDBORNE MYCOFLORA OF SUNFLOWER

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Abstract

Using blotter, agar plate and deep-freezing methods as recommended by ISTA, the seedborne mycoflora of sunflower was shown to consist of 20 different genera and 36 species of fungi. More fungi were isolated by the blotter method followed by agar plate and the deep-freezing method. Variety HO-1 showed highest fungal colonization among the 20 samples tested. The pathogenic species included *Rhizoctonia solani* Kuhn, *Macrophomina phaseolina* (Tassi) Goid, *Fusarium moniliforme* Sheldon, *F. solani* (Mart.) Appel & Wollenw., *F. semitectum* Berk. & Rav., *F. equiseti* (Corda) Sacc., *Drechslera* state of *Cochliobolus spicifer* Nelson, *Alternaria alternata* (Fr.) Keissler, *A. tenuissima* (Kunze ex Pers.) Wiltshire, whereas storage fungi such as *Aspergillus flavus* Link., and *A. niger* van Tieghem dominated. Surface sterilization of seeds with 1% $Ca(OCl)_2$ re duced the colonization of *Aspergillus* species and *R. solani* whereas *M. phaseolina* and *Fusarium* species showed an increase.

Introduction

Sunflower (*Helianthus annuus* L.) is an oil seed crop planted over 25,899 hectares in Pakistan (Anon., 1990). It is assuming increasing importance because the seed contain 25-32% edible oil which is a rich source of polyunsaturated fatty acids used for human consumption (Neergaard, 1977). Of the various disease causing organisms, fungi are known to decrease the quantity and quality of seed (Singh & Prasad, 1977). For example *Plasmopara halstedii* (Farl.) Berl. & de Toni occurs in the seed coat and infected seed show low germinability and produce abnormal seedlings (Zad, 1978). Seed infected with *P. halstedii* and *Puccinia helianthi* Schw. are significantly smaller, lighter, higher in hull percentage and low in oil content (Zimmer & Zimmerman, 1972). Infection of seeds with *Alternaria alternata* (Fr.) Keissler *Aspergillus flavus* decreased the oil and iodine content of seeds (Prasad, 1983). Except for the reports of *Alternaria zinnae* Pape (Khan *et al.*, 1974) and *Curvularia lunata* (Wakker) Boed., (Ghafoor, 1974), there does not appear to be any report on the seedborne mycoflora of sunflower in Pakistan. In addition, since fungi are known to produce mycotoxins (Rodricks, 1976), it is important to know the composition of the mycoflora of sunflower seeds. The results of our studies on the seedborne mycoflora of sunflower are presented in this paper.

Materials and Methods

Twenty samples of sunflower seed collected from Sindh (15), Punjab (2), NWFP (1), Balochistan (1) and Kashmir (1) were used to detect the seedborne mycoflora.

Table 1. Seed borne mycoflora of sunflower.

	Surface Sterilized Seeds						Non Sterilized Seeds					
	Blotter		Agar plate		Deep freezing		Blotter		Agar plate		Deep freezin	
	NSI	I%±SD	NSI	I%±SD	NSI	I%±SD	NSI	I%±SD	NSI	I%±SD	NSI	I%±SD
Alternaria alternata	12	14.0±13.00	6	13.5±10.4	4	5.5±2.5	9	12.34±9.3	5	6.6±4.7	3	4.1±1.4
**A. tenuissima*	2	10.1±3.05	2	5.5±1.3	2	6.9±1.9	1	3.8±0.85	2	6.03±1.4	2	9.4±2.8
**Aspergillus amsteldomi*	1	0.67±0.15	1	1.3±0.3	-	——	-	——	-	-----	-	------
A. candidus	7	4.6±1.9	1	0.4±0.1	1	0.22±05	6	8.6±5.5	2	0.34±0.1	-	------
A. flavus	14	10.35±8.2	14	9.0±6.0	7	6.0±5.0	15	21.5±12.8	15	11.0±10.0	10	6.0±4.0
A. fumigatus	2	0.9±0.3	3	0.80±0.3	-	——	-	-----	1	0.67±0.15	2	0.6±0.2
A. niger	15	8.3±7.1	16	16.0±15.0	6	1.6±1.0	12	14.5±9.07	7	20±17.0	4	3.5±1.6
**A. quadrilineatus*	3	1.6±1.15	1	0.4±0.1	1	5.3±1.2	-	0.6±0.15	-	------	1	1.1±.25
**A. sulphureus*	3	1.1±.45	1	0.89±0.2	-	------	2	0.78±0.25	1	0.4±0.1	-	------
A. terreus	4	2.2±1.0	3	0.7±0.2	-	------	4	3.3±3.0	5	2.6±1.5	-	------
**A. wentii*	3	2.8±1.0	3	2.4±0.9	-	——	5	7.1±2.2	7	2.5±1.9	-	------
**Botryodiplodia theobromae*	2	1.4±0.4	2	1.2±0.3	-	-----	2	3.1±0.8	1	0.4±0.1	-	-----
**Cephaliophora irregularis*	-	-----	-	------	-	------	1	0.81±0.2	-	------	-	-------
**Cephalosporium* sp.	1	0.4±0.1	-	-----	-	------	-	------	-	------	-	------
**Chaetomium crispetum*	1	0.4±0.1	-	-----	-	------	-	------	-	------	-	-------
C. globosum	3	1.5±0.55	7	3.5±1.9	7	7.9±4.5	2	1.35±0.35	4	1.9±0.8	6	9±5.7
Curvularia lunata	-	-----	1	0.48±.15	-	-----	1	1.1±.25	1	0.22±.05	-	-----
**Drechslera australiensis*	-	------	3	1.66±0.5	-	-----	-	-----	1	0.22±.05	-	-----
**D.* state of *Cochliobolus spicifer*	1	4.0±0.9	-	-----	-	-----	2	1.08±.35	2	1.08±0.35	-	-----
**Fusarium equiseti*	1	1.78±0.4	-	-----	1	1.78±0.4	1	0.89±0.2	-	-----	1	0.89±0.2

Table 1 (Cont'd)

	Surface Sterilized Seeds						Non Sterilized Seeds					
	Blotter		Agar plate		Deep freezing		Blotter		Agar plate		Deep freezin	
	NSI	I%±SD	NSI	I%±SD	NSI	I%±SD	NSI	I%±SD	NSI	I%±SD	NSI	I%±SD
**F. heterosporum*	-	-----	-	------	-	------	1	0.4±0.1	1	0.4±0.1	-	-----
F. moniliforme	4	1.1±0.5	4	3.4±2.7	6	4.5±2.4	4	3.25±1.89	3	0.36±.15	5	2.05±.85
**F. semitectum*	1	0.4±0.1	-	-----	1	0.89±0.2	-	-----	-	-----	-	-----
**F. solani*	3	3.1±.85	3	2.25±.65	4	5±3.75	1	0.4±0.1	-	-----	3	1.6±0.57
Macrophomina phaseolina	2	5.0±1.4	2	4.5±3.5	1	0.22±.05	-	-----	1	.22±.05	-	------
**Melanospora* sp.	1	0.4±0.1	-	-----	1	0.65±0.15	1	0.2±.05	-	-----	-	-----
Mucor sp.	-	-----	-	-----	-	------	1	0.8±0.2	-	-----	-	------
Penicillium sp.	3	1.79±0.5	-	-----	-	------	2	3.2±.9	1	0.2±.05	1	31±0.7
**Rhizoctonia solani*	1	2.9±0.65	1	4.4±1.0	1	0.2±.05	1	4.6±1.0	5	5.5±1.25	-	------
Rhizopus sp.	9	11.5±7.5	-	------	-	------	9	15.6±6.9	1	0.89±0.2	-	-----
**Scopulariopsis brevicaulis*	-	-----	1	2.2±0.5	-	-----	-	0.89±0.2	1	0.2±.05	1	0.4±0.1
**Stachybotrys atra*	-	-----	-	-----	-	------	1	0.89±0.2	-	------	-	------
**Stemphylium* state of *Pleospora herbarum*	1	0.89±0.2	-	-----	-	-----	-	------	-	-----	-	------
**Trichoderma* sp.	-	-----	-	------	1	0.89±0.2	-	-----	1	1.1±0.25	1	1.1±0.25
**Trichothecium roseum*	1	7.8±1.75	-	0.89±0.2	1	0.89±0.2	1	11.6±2.6	1	3.8±0.85	1	3.35±0.75
*Unidentified ascomycetes	-	-----	-	------	-	-----	-	------	1	1.1±0.25	-	------

NSI# = Number of samples infected out of 20 samples tested. * = New reports on sunflower seeds.
I%±SD = % of infected seed. ± standard deviation

Using ISTA technique (Anon., 1976) 400 seeds from each sample were tested. For the standard blotter technique, untreated seed and seed after treatment with 1% $Ca(OCl)_2$ were placed on three layers of moistened blotters, 10 seeds per Petri dish. For the agar plate method, the treated and untreated seeds were plated on potato dextrose agar (PDA), 10 seeds per Petri dish and the dishes were incubated at 24oC in alternating cycle of 12h light and 12 h darkness for 7 days. In the deep- freezing method, the treated and untreated seed were incubated for 1 day each at $20^{o}C$ and at $0^{o}C$ in a freezer followed by 5 days incubation at $24^{o}C$ and fungi growing on seeds were identified after reference to Barnett (1960), Booth (1971), Ellis (1971), Gilman (1957), Nelson *et al.*, (1983) and Raper & Fennel (1965).

Results and Discussion

Of the total number of 20 genera and 36 species of fungi isolated (Table 1) 23 different species viz., *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire, *Aspergillus wentii* Wehmer, *A. amsteldomi* (Mangin) Thom & Church, *A. quadrilineatus* Thom & Raper, *A. sulphureus* (Fres.) Thom & Church, *Botryodiplodia theobromae* Pat., *Cephaliophora irregularis,* Thaxter, *Cephalosporium* sp., *Chaetomium crispetum* Fuckel, *Drechslera australiensis* (Bugni.) Subram. & Jain ex M.B. Ellis, *D.* state of *Cochliobolus spicifer* Nelson, *Fusarium equiseti* (Corda) Sacc., *F. heterosporum* Nees, *F. semitectum* Berk and Rav., *F. solani* (Mart.) Appel & Wollenw., *Melanospora* sp., *Rhizoctonia solani* Kuhn, *Scopulariopsis brevicaulis* Bain, *Stachybotrys atra* Corda, *Stemphylium* state of *Pleospora herbarum* (Pers. ex Fr.) Rabenh, *Trichoderma* sp., *Trichothecium roseum* Link and an unidentified ascomycetes appear to be new report on sunflower seed (Richardson, 1979, 1981, 1983). These species have been marked with an asterisk in Table 1. At least 5% of the seed samples were infected by *R. solani* with an infection percentage of 13.0 for surface sterilized seeds, whereas 60% of the seed samples were infected by *Alternaria alternata* with an infection range of 1-27%. *A. flavus* Link., and *A. niger* dominated with 75 and 65% of the samples infected by these two species. There was an infection range of 9-38% and 4-24% respectively, in non-sterilized seed. Surface sterilization of seed with 1% $Ca(OCl)_2$ reduced the incidence of *Aspergillus* spp., and *R. solani*, whereas *M. phaseolina* and *Fusarium* spp., showed an increase. Since microbial contamination is eliminated by chlorine disinfection (Limonard, 1968), it would appear that several of the fungi can be deep-seated but most often are superficially borne. Of the 20 samples tested, variety HO-1 from Sindh which is an open pollinated variety with an average yield of 2.2 mt/hectare and 40% oil content (Chaudhary, 1988), showed the highest incidence of fungi. Sixteen species were isolated from this variety compared to samples from Punjab, Balochistan, NWFP and Kashmir.

Of the three different methods used, the blotter technique yielded the highest number of fungi as compared to the Agar plate and deep-freezing methods. Similar results have been observed by Khan *et al.*, (1988) on rice seed. The deep-freezing method was superior to the standard blotter and agar plate methods for the detection of *Fusarium* spp., *Chaetomium globosum* Kunze ex Staud., and *A. quadrilineatus*. Mathur *et al.*, (1975) working with sorghum seed also found that the deep-freezing

method was more suitable for the detection of *Fusarium* spp. Similar results have been reported for *Fusarium* spp., *Drechslera* sp., *Curvularia* sp., *Trichoconis padwickii* Ganguly, and *Myrothecium roridum* Tode ex Fr. in seed of sorghum (Sultana *et al.*, 1988a), chillies (Sultana *et al.*, (1988b) and rice (Khan *et al.*, 1988).

A number of the fungi isolated in the present study especially those in the genera *Aspergillus* and *Fusarium* are known to be potent mycotoxin producers. Mycotoxins can cause severe damage to the liver, kidneys and nervous system of man even in low dosages (Rodricks, 1976). Aflatoxin B_1, B_2, and G_1, G_2 are the four major metabolites of *A. flavus* and *A. parasiticus* which are hepato-carcinogenic (Diener & Davis, 1969). Ochratoxin produced by *A. ochraceus* and citrinin produced by *A. terreus* affect the kidneys. Zearalenone produced by *F. moniliforme* affects the uterus (Martin & Gilman, 1976; Rodricks, 1976), whereas *F. equiseti* metabolites can cause haemorrhage, exhaustion, diarrhoea and weight loss (Diener & Davis, 1969). There is therefore, a need for the study of the mycoflora of sunflower seed kept under different storage conditions before it is used for human consumption.

References

Anonymous. 1976. International rules of seed testing. *Proc. Int. Seed Test. Assoc.*, 4: 3-49.

Anonymous. 1990. *Agriculture statistics of Pakistan.* 1989-1990. Govt. of Pakistan, Ministry of Food and Agriculture and Co-operatives. Food and agriculture Division (Economic Wing), Islamabad. pp. 316.

Barnett, H.L. 1960. *Illustrated genera of Imperfect Fungi.* (Second Edition). Burgess Pub. Co., pp.225.

Booth, C. 1971. *The genus Fusarium.* CMI, Kew, Surrey, England. pp. 237.

Chaudhary, A.H. 1988. Highlights of improvement in research on oil seed crops in Sind. Biland Rai. Oad and Kirshan Mehraj oil seeds Department, Agriculture Research Institute, Tandojam. pp. 40.

Diener, U.L. and N.D. Davis. 1969. Relation of environment to aflatoxin production from *Aspergillus flavus.* pp. 15-34. In: *Aflatoxin,* (Ed.) L.A. Goldblatt. Academic Press, New York.

Ellis, M. B. 1971. *Dematiaceous Hyphomycetes.* CMI, Kew, Surrey, England. pp. 608.

Ghafoor, A. and S.A.J. Khan. 1974. *List of diseases of economic plants in Pakistan.* Ministry of Food and Agriculture. pp. 85.

Gilman, J.C. 1957. *Manual of soil fungi* (Second edition). The Iowa State University Press, Ames., Iowa, USA. pp.450.

Khan, S.A.J, A.K. Khanzada, N. Sultana and M. Aslam. 1988. Evaluation of seed health testing techniques for the assessment of seed borne mycoflora of rice. *Pakistan J. Agric. Res.*, 9: 502-505.

Limonard, T. 1968. Ecological aspects of seed health testing. *Proc. Int. Seed Test. Assoc.*, 33: 343-513.

Martin. P.M.D. and G.A. Gilman. 1976. A consideration of the mycotoxin hypothesis with special reference to the mycoflora of maize, sorghum, wheat and groundnuts. *Rep. Trop. Prod. Inst.*, G105.1.

Mathur, S.K., S.B. Mathur and P. Neergaard. 1975. Detection of seed borne fungi in Sorghum and location of *Fusarium moniliforme* in seed. *Seed Sci. and Technol.*, 3: 683-690.

Neergaard, P. 1977. *Seed Pathology.* Volume 1. The MacMillan Press Ltd. , London. pp. 839.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium species.* An Illustrated Manual for Identification. The State Univ. Press. pp. 193.

Prasad, T. and B.K. Singh. 1983. Effect of relative humidity on oil properties of fungal infested sunflower seeds. *Biological Bulletin of India*, 5: 85-88.

Raper, K.E. and D.I. Fennel. 1965. *The genus Aspergillus.* The Williams and Wilkins Company, Baltimore. pp. 686.

Richardson, M.J. 1979. *An Annotated List of Seed Borne Diseases.* Int. Seed Test. Assoc., Zurich, Switzerland. pp. 320.

Richardson, M.J. 1981. *An Annotated List of Seed Borne Diseases.* Suppl. I. Int. Seed Test. Assoc., Zurich, Switzerland, pp. 78.

Richardson, M.J. 1983. *An Annotated List of Seed Borne Diseases.* Suppl. II. Int. Seed Test. Assoc., Zurich, Switzerland, pp. 108.

Rodricks, J.V. 1976. *Mycotoxins and other fungal related food problems.* Advances in Chemistry Series 149. American Chemicals Society, Washington.

Singh, B.K. and T. Prasad. 1977. Effect of seed borne fungi on the Physico-Chemical properties of sunflower oil. *Phytopath.* Z.,90: 337-341.

Sultana, N., S.A.J. Khan and A.K. Khanzada. 1988. Detection and location of seed borne fungi in sorghum seed. *Pak. J. Bot.*, 20: 239-244.

Sultana, N., S.A.J. Khan and A.K. Khanzada. 1988. Studies on seed borne mycoflora of chillies and control of fruit rot disease. *Pak. J. Sci. Ind. Res.*, 31: 365-368.

Zad, J. 1978. Transmission of sunflower downy mildew by seed. *Iranian Journal of Plant Pathology,* 14: 1-2.

Zimmer, D.E. and D.C. Zimmerman. 1972. Influence of some diseases on achene and oil quality of sunflower. *Crop Science,* 12: 859-861.

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