Pak.J.Bot., 27(1): 201-208, 1995.

SEEDBORNE MYCOFLORA OF TOMATO

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Abstract

Using ISTA techniques, the seedborne mycoflora of 24 samples of tomato seeds collected from different parts of Pakistan was examined. Of the 37 species of fungi belonging to 20 genera isolated, 22 species of fungi belonging to 15 genera were found to be new records from Pakistan. *Fusarium solani, F. moniliforme, Aspergillus flavus, Alternaria alternata* and *Drechslera australiensis* were predominant. Greater number of fungal species, both in sterilized and non-sterilized seeds, were recorded by agar plate method followed by blotter and deep-freezing method.

Introduction

Tomato (*Lycopersicon esculentum* Mill.), an important vegetable crop is cultivated over an area of 20,3835/hectares in Pakistan with an a average production of 213,534 tons/year (Anon, 1991). It is known to suffer from a number of diseases which adversely affect crop production. Some of these diseases are transmitted through seeds. (Richardson, 1979,1981,1983). Atleast 15 fungi have been reported as seedborne on tomato from Pakistan (Ahmed *et al.*, 1993) whereas 10 fungi have been reported from different parts of the world (Richardson, 1979, 1981, 1983). Experiments were carried to study the seedborne mycoflora of tomato seed samples collected from different parts of Pakistan.

Materials and Methods

Twenty four samples of tomato seeds collected from different parts of Pakistan like Karachi, Islamabad, Lahore, Quetta, Mirpurkhas and Faisalabad were used to analyse the seed borne mycoflora using ISTA techniques (Anon., 1976). From each sample 400 seeds were used. For the standard blotter technique, non-sterilized seeds and seeds sterilized with 1% $Ca(OCl)_2$ for 5 minutes were placed on three lyers of moistened blotter, 10 seeds per Petri dish. For Agar plate method, the sterilized and non-sterilized seeds were plated on potato dextrose agar (PDA), 10 seeds per Petri dish, and the dishes were incubated at 24°C for 7 days under a 12h alternating cycle of light and darkness. In the deep-freezing method, the sterilized and non-sterilized seeds were placed on three lyers of moistened blotter and incubated for 1 day each at 20°C and at 0°C in a freezer followed by 5 days incubation at 24°C. Fungi isolated from seeds were identified after reference to Barnett (1960), Booth (1971), Ellis (1971), Gilman (1957), Nelson *et al.*, (1983) and Raper & Fennel (1965).

Table 1. Incidence of fungi in tomato seeds as tested by three incubation methods. (Observation based on 400 seeds used for testing in each method).

	Non-sterilized seeds						Sterilized seeds					
	BLOTTER		AGAR PLATE		DEEP-FREEZING		BLOTTER		AGAR PLATE		DEEP-FREEZING	
Name of Fungus	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD
Alternaria alternata	17	6.0±5.6 (4-20)	16	5.77±5.35 (2-15.5)	15	1.66±1.85 (1-7)	18	5.06±3.85 (3-12.5)	15	4.22±4.47 (2-18.5)	16	2.25±1.98 (1-6.5)
A. tenuissima	7	3.81±9.19 (3-42)	6	2.29±5.01 (1-18)	4	0.31±0.74	8	2.20±3.59 (3-10.5)	5	1.29±3.05 (1-11)	4	0.52±1.24 (2-4.5)
Aspergillus candidus	0	--- ---	2	0.25±0.89 (2-4)	0	--- ---	1	0.12±0.61 (0-3)	1	0.04±0.20 (0-1	1	0.02±0.10 (0-0.5)
A. flavus	18	8.12±6.35 (3-20)	22	12.29±7.95 (4-29)	18	5.72±9.61 (2-27)	20	5.72±5.57 (2-25)	23	8.50±6.31 (2-23)	17	4.93±6.99 (2-27)
A. fumigatus	0	--- ---	0	--- ---	0	--- ---	0	--- ---	2	0.16±0.56 (0-2)	0 0	--- ---
A. niger	12	2.66±3.29 (2-9)	23	15.50±8.85 (2.33)	4	0.50±1.38 (1-6)	12	1.62±1.92 (1-6)	21	6.14±4.77 (2-19)	3	0.66±1.89 (3.5-7.5)
A. guadrilineatus	5	1.00±2.50 (1-10.5)	10	2.20±3.18 (3-12)	4	0.41±1.07 (2-4)	2	0.45±1.17 (1-4)	10	1.77±2.34 (4-6.5)	4	1.97±5.07 (1-4)
A. sulphureus	1	0.04±0.20 (0-2)	2 2	0.18±0.67 (1.5-3)	0 0	--- ---	1 1	0.08±0.40 0.08±0.40	2 2	0.20±0.72 0.20±0.72	0 0	--- ---
A. terreus	4	0.45±1.14 (1-4)	6 6	1.00±2.06 (2-8)	2	0.16±0.63 (1-3)	3	0.29±1.04 (1-5)	8	1.08±1.89 (1-61)	3	0.25±0.69 (1.5-2.5)
A. wentii	0	---	0	--	2	1.20±4.87 (5.5-23.5)	1	0.04±0.20 (0-1)	0	---	2 2	0.77±3.37 (2-16.5)

Table 1 (Cont'd.)

Name of Fungus	Non-sterilized seeds						Sterilized seeds					
	BLOTTER		AGAR PLATE		DEEP-FREEZING		BLOTTER		AGAR PLATE		DEEP-FREEZING	
	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD
A. sp.	0	---	3	1.41±4.74 (2-21)	1	0.50±2.44 (0-12)	1	0.168±0.81 (0-4)	6	1.62±3.48 (1-13)	2	1.02±3.49 (10.5-14.0)
*Auriobasidium pullulans	0	---	0	---	0	---	0	---	1	0.12±0.61 (0-3)	0	---
*Cephaliophora irregularis	0	---	2	0.29±1.08 (2-5)	0	---	0	---	1	0.39±1.93 (0-9.5)	0	---
Chaetomium globosum	14	3.10±3.35 (2-13)	8	2.10±4.55 (2-19)	3	0.72±2.07 (3.5-8)	17	4.93±4.92 (2-18)	15	5.22±8.96 (2-39)	4	1.08±2.81 (2-11)
*C. indicum	5	0.68±1.45 (2-4.5)	2	0.41±1.41 (0-5)	1	0.08±0.40 (0-2)	5	0.83±1.97 (2-8)	5	0.95±2.25 (2-9)	1	0.29±1.42 (0-7)
Cladosporium sp.	6	1.02±2.15 (1-8)	8	1.79±3.16 (2-10)	1	0.16±0.81 (0-4)	4	0.87±2.75 (2-13)	12	1.62±2.49 (1-11)	2	0.16±0.63 (1-3)
Curvularia lunata	5	1.45±2.51 (2-8.5)	6	1.221±2.52 (2-9)	1	0.14±0.71 (0-3.5)	4	0.62±1.55 (2-1)	7	1.18±2.34 (2-9)	1	0.08±0.40 (0-2)
*C. tuberculata	0	-	0	-	0	-	0	-	3	0.33±0.91 (2-3)	0	---
*Drechslera australiensis	12	3.37±4.02 (2-12)	7	4.916±4.828 (2-13)	5	0.833±1.094 (1.5-3.0)	6	2.083±2.810 (2.5-9.5)	4	3.625±5.928 (2-16)	0	-
*D. hawaiiensis	0	--- ---	1	0.41±2.04 (0-10)	0	---	0	---	0	---	4	1.041±2.435 (1-8.5)

Table 1 (Cont'd.)

Name of Fungus	Non-sterilized seeds						Sterilized seeds					
	BLOTTER		AGAR PLATE		DEEP-FREEZING		BLOTTER		AGAR PLATE		DEEP-FREEZING	
	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD
D. state of Cochliobolus spicifer	1	0.08±0.40 (0-1)	1	0.10±0.51 (0-2.5)	0	-	1	0.31±1.53 (0-7.5)	0	--- -	0	--- ---
Fusarium moniliforme	11	3.979±5.86 (2-23)	7	2.91±8.06 (2.5-39)	9	1.27±2.28	13	4.62±6.90 (2-31)	12	3.33±4.06 (2-12)	8	1.25±1.93 (2-6)
F. oxysporum	10	2.58±4.56 (2-20)	9	3.52±8.58 (2-41)	5	0.62±1.58 (1-7)	10	3.37±5.07 (3-11)	13	4.50±7.40 (2-33)	8	0.75±1.21 (1-4)
**F. semitectum*	0	---	0	---	1	0.25±1.22 (0-6)	0	---	0	---	---	0.04±0.20 (0-1)
F. solani	16	6.22±7.90 (3-36)	14	5.25±5.93 (2.5-21)	6	0.72±1.52 (1.5-6.0)	19	8.47±10.57 (3-41)	16	6.60±7.53 (4-34)	11	1.66±2.16 (1-6)
**Mucor* sp.,	3	0.25±0.73 (1-3)	1	0.12±0.61	0	--- (0-3)	0	---	0	---	0	---
**Macrophomina phaseolina*	1	0.18±0.91) (0-4.5)	1	0.04±0.20 (0-1)	0	--- ---	0	--- ---	1	0.125±0.612 ---	0	---
Nigrospora oryzae	4	0.64±1.52 (3-5)	5	0.83±1.83 (2-7)	2	0.12±0.44 (1-2)	4	0.50±1.17 (2-4)	6	0.95±1.92 (1-6)	2	0.12±0.44 (1-2)
Penicillium sp.,	6	2.02±6.94 (2-34)	11	4.37±11.78 (2-58)	4	2.47±8.51 (1.5-40)	6	2.08±7.22 (1-35)	9	2.95±10.10 (1.5±50)	3	1.27±5.12 (1.5-25)
**Phoma* sp.	0	---	0	---	---	---	0	---	1	0.06±0.30 (0-1.5)	0	---

Table 1 (Cont'd.)

Name of Fungus	Non-sterilized seeds						Sterilized seeds					
	BLOTTER		AGAR PLATE		DEEP-FREEZING		BLOTTER		AGAR PLATE		DEEP-FREEZING	
	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD	NSI	% of infected seed ± SD
Paecilomyces lilacinus	0	---	2	0.22±0.80 (2-3.5)	0	---	1	0.18±0.91 (0-4.5)	1	0.06±0.30 (0-1.5)	0	---
P. varioti	0	---	1	0.16±0.81 (0-4)	0	---	0	---	3	0.35±1.02 (1.5-4)	0	---
Rhizopus sp.,	7	1.70±3.00 (2.5-9.5)	6	2.04±4.35 (2.5-16)	0	---	0	---	0	---	0	---
Scopulariopsis sp.	0	---	0	---	0	---	0	---	1	0.12±0.61 (0-3)	0	---
Stachybotrys atra	2	0.66±2.42	0	---	0	---	2	0.33±1.16 (3-5)	1	0.10±0.51	0	--- (0-2.5)
Syncephalastrum sp.	0	---	1	0.14±0.71	0	---	0	---	0	---	0	---
Trichoderma harzianum	0	---	4	1.41±4.77	0	--- (0-4)	0	---	4	1.14±2.87 (3.5-11)	1	0.29±1.42 (0-7)

NSI = No. of sample infected

SD = Standard deviation. (Number in parenthesis indicate infection range)

Results and Discussion

A total of 37 species of fungi belonging to 20 genera viz., *Alternaria alternata, A. tenuissima, Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. quadrilineatus, A. sulphureus, A. terreus, A. wentii, A.* sp., *Auriobasidium pullulans, Cephaliophora irregularis, Chaetomium globosum, C. indicum, Cladosporium* sp., *Curvularia lunata, C. tuberculata, Drechslera australiensis, D. hawaiiensis, D.* state of *Cochliobolus spicifer, Fusarium moniliforme, F. oxysporum, F. semitectum, F. solani, Mucor* sp., *Macrophomina phaseolina, Nigrospora oryzae, Penicillium* sp., *Paecilomyces varioti, P. lilacinus, Rhizopus* sp., *Scopulariopsis* sp., *Stachybotrys atra, Syncephalastrum* sp., *Trichoderma harzianum* and an unidentified ascomycetes were isolated and identified.

Of these atleast 20 species of fungi viz., *Aspergillus candidus* Link., *A. fumigatus* Fres., *A. quadrilineatus* Thom & Raper, *A. sulphureus* (Fres.) Thom & Church, *A. terreus* Thom, *A. wentii* Wehmer, *Auriobasidium pullulans* De Bary, *Cephaliophora irregularis* Thaxter, *Chaetomium indicum* Corda, *Curvularia tuberculata* Jain, *Drechslera australiensis, D. hawiiensis* (Bugni). Subram & Jain ex. M.B. Ellis, *Fusarium semitectum* Berk & Rav., Mucor sp., *Macrophomina phaseolina* (Tassi) Goid., *Phoma* sp., *Paecilomyces varioti* Bainier, *Scopulariopsis* sp., *Stachybotrys atra* Corda, *Syncephalastrum* sp., and *Trichoderma harzianum* Pers. do not appear to have been previously reported on tomato seeds (Richardson, 1979, 1981, 1983; Iftikhar, 1993).

Seed samples collected from Karachi showed maximum infection of *Alternaria alternata* (4 to 20%), *Chaetomium globosum* (2 to 39%), *Fusarium solani* (3 to 41%) *F. oxysporum* (2 to 41%) whereas *Penicillium* sp., (2-56%) was found preponderant in seed samples collected from Quetta. *Phoma* sp., *Scopulariopsis* sp., and *Syncephalastrum* sp., were isolated in low frequency showing an average infection of 1.5, 3.0 and 3.5%, respectively.

Of the 3 methods used for isolation of fungi, blotter method was found more suitable for isolation of *Alternaria* spp., *Fusarium* spp., and *Drechslera* spp. Neergaard (1977) also reported that blotter method provides better conditions for conidial sporulation of many Fungi Imperfecti like *Alternaria, Drechslera* and *Fusarium* spp. Agar plate method was found suitable for the isolation of *Aspergillus* spp., *Curvularia* spp., *Trichoderma* sp., *Penicillium* sp., and *Fusarium oxysporum* as also reported for the isolatium of fast growing saprophytes (Limonard, 1968; Tempe, 1970). Deep-freezing method yielded minimum numbers of *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. It is interesting to note that Deep-freezing method has been reported to be more suitable for the detection of *Fusarium* spp., on sorghum seeds (Mathur *et al.*, 1975), chilies (Sultana *et al.*, 1988b) and rice (Khan *et al.*, 1988). In the present study infection of *Fusarium* spp., greatly reduced where deep-freezing method was used. Deep-freezing method presumably eliminated externally seedborne fungi which also affected the internally seed borne infection by *Fusarium* spp.

Greater incidence of *Chaetomium* spp., *F. oxysporum* and *F. solani* was observed after surface sterilization of seeds with 1% $Ca(OCl)_2$. It would therefore suggest that *Chaetomium* spp., and *Fusarium* spp., were mostly internally seedborne as compared to

other fungi which were presumably externally seedborne. Removal of externally seed borne fungi by surface sterilization provided a chance for the internally seedborne fungi to appear in greater number. There are reports that *F. oxygsporum* remained viable in dried pulp fragment on the surface of seeds for many years (Besri, 1977, 1978; Frisullo, 1988). The isolation of *F. oxysporum* from greater number of seed samples after surface sterilization would suggest that *F. oxysporum* was also internally seed borne. The results of the present study shows that tomato seeds are infected with pathogenic fungi like *Alternaria alternata, Fusarium oxysporum* and *F. moniliforme* that are known to produce seed rot, root rot and wilt diseases in tomato (Franceschini, 1982; Huang & Sun, 1982; Perveen & Ghaffar, 1991). There is therefore need for the control of seed borne mycoflora for better germination of seeds and control of seedborne diseases.

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(Received for Publication 25 September 1993)