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SEED HEALTH TESTING OF CUCUMBER (*CUCUMIS SATIVUS* L)

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Using ISTA techniques, the seed-borne mycoflora of cucumber (*Cucumis sativus* L.) was studied. The blotter method was found to be the most suitable technique for detection of fungi in cucumber seed. Deep freezing method was preferable for the detection of *Fusarium* spp. A total of 18 genera and 33 species were isolated, of which 25 have not hitherto been recorded from seeds of cucumber in Pakistan.

Key words: Cucumber, Mycoflora, ISTA technique.

Introduction

Cucumber (*Cucumis sativus* L.) is the most popular and widely cultivated cucurbitaceous vegetable crop of Pakistan. Some of the seed-borne fungi on cucumber in Pakistan are *Alternaria* spp., *Aspergillus* spp., *Curvularia* spp., *Fusarium equiseti, F. moniliforme, Macrophomina phaseolina, Myrothecium roridum, Penicillium* spp. and *Rhizopus* spp. (Ahmad *et al* 1993). Reports of the fungi dominant on cucurbit seeds include *Macrophomina phaseolina, Fusarium equiseti, F. solani* and *F. semitectum* (Sheikh 1990). The present study describes the efficiency of laboratory techniques for detection of seed-borne fungi of cucumber.

Materials and Methods

Sixteen samples of cucumber seeds collected from different places in Sindh, Balochistan and Punjab during the crop season 1994-95 were analyzed for seed-borne mycoflora by using ISTA techniques (Anon 1976). For standard blotter and agar plate methods, seeds were tested before and after treatment with 1% NaOCl for 5 min and were placed on three layers of moistened blotters (20 seeds per 9 cm diameter petri dish) and on potato dextrose agar (PDA) (5 seeds per 9 cm diameter petri dish). The dishes were incubated at 24 ± 1°C under 12 h alternating cycles of ADL (Artificial Day Light supplied by cool white fluorescence tube) and darkness for 7 days. In the deep freezing method the treated and untreated seeds were incubated for 1 day each at 20°C and -20°C followed by 5 days incubation at 24±1°C under 12 h alternating cycles of ADL and darkness. Fungi growing on seeds were identified with reference to Barnett and Hunter (1977), Booth (1971), Ellis (1971), Nelson *et al* (1983) and Raper and Fennel (1965).

Results and Discussion

Three testing methods yielded 18 genera and 33 fungal species from sixteen seed samples of cucumber collected from different parts of Pakistan (Table 1). Of these 25 fungal species do not appear to have been recorded previously from seeds of cucumber in Pakistan. The blotter method revealed more fungal species (33) followed by the deep freezing method (24) and agar plate method (20). The standard blotter method was reported to be the best for the detection of seed-borne fungi in different crops (Bhutta 1988; Chraya and Ready 1979). Germination range was from 54-85% in the seed samples tested.

The average percent incidence and the range of occurrence of fungi in seed samples tested revealed that *Cladosporium cladosporioides, Fusarium moniliforme, F. oxysporum* and *Macrophomina phaseolina* were the most frequent in cucumber seeds. The deep freezing method yielded maximum count of *Fusarium* spp. and *Myrothecium roridum*. This finding corroborates the reports that the deep freezing method is more suitable for deeply seated seed-borne fungi especially for *Fusarium* spp. (Mathur *et al* 1975; Khan *et al* 1988; Dickmann and Assend 1989; Rujirachoon 1998; Sultana *et al* 1992).

Disinfestation of the seeds with 1% sodium hypochlorite lowered the incidence of *Aspergillus spp., Cladosporium* spp. and *Rhizopus* spp. whereas these ubiquitous fungi were isolated in higher percentage using the agar plate method. *Curvularia lunata* and the *Drechslera* state of *Cochliobolus spicifer* were isolated in higher percentage on agar plate method where disinfected seeds of cucumber were used. Chlorine pretreatment increased the recovery percentage of *Alternaria* spp., *Fusarium* spp., *Macrophomina phaseolina* and *Myrothecium roridum*. These observations are in close agreement with the

Table 1

Incidence of fungi in cucumber seeds tested by three incubation methods. (Observations based on 400 seeds used for testing in each method).

Fungi	Blotter			Deep freezing			Agar plate		
	SI	Control	Treated	SI	Control	Treated	SI	Control	Treated
*Alternaria alternata** (Fr.) Keissler	10	6.6±0.3 (0.5-12.0)	7.1±0.5 (2.5-14.0)	10	6.1±0.5 (2.0-10.0)	6.8±0.2 (4.0-12.8)	8	2.1±0.2 (0.3-3.5)	5.8±0.4 (1.0-8.0)
*A. tenuissima** Kunze ex Pers	1	--	3.5±0.0 (3.5)	--	--	--	--	--	--
*Aspergillus candidus** Link	1	4.0±0 (4.0)	3.0±0.0 (3.0)	1	5.5±0.0 (5.5)	2.0±0.0 (2.0)	1	8.5±0.0 (8.5)	4.3±0.0 (4.3)
*A. flavus** Link & Pers.	12	4.7±0.3 (2.5-5.5)	3.1±0.3 (1.0-4.0)	12	4.1±0.4 (2.0-5.0)	1.3±0.2 (0.5-2.0)	12	15.2±0.7 (6.5-24.0)	7.9±0.4 (2.0-15.0)
*A. niger**Van Tiegh	13	5.7±0.4 (0.5-12.5)	4.3±0.3 (3.5-5.5)	12	6.9±0.7 (1.0-13.0)	5.3±0.6 (0.5-8.0)	14	14.8±0.9 (5.0-49.0)	7.8±0.6 (1.0-30.5)
*A. terreus** Thom.	3	1.2±0.3 (0.5-2.0)	0.5±0.0 (0.5)	2	1.4±0.6 (0.5-2.3)	0.4±0.1 (0.3-0.5)	3	2.8±0.4 (2.0-4.2)	2.0±0.4 (1.5-2.5)
*A. wenteii** Wehmer	1	2.5±0.0 (2.5)	1.0±0.0 (1.0)	1	1.5±0.0 (1.5)	0.8±0.0 (0.8)	1	7.0±0.0 (7.0)	3.0±0.0 (3.0)
*Chaetomium funicola** Cooke	6	6.4±0.4 (2.0-12.0)	2.6±0.2 (0.5-4.0)	--	--	--	4	2.1±0.2 (0.5-4.0)	2.6±0.4 (2.0-2.8)
*C. globossum** Kunze ex Fr.	10	4.3±0.3 (3.0-7.0)	2.1±0.3 (0.5-3.0)	2	1.0±0.4 (0.5-1.5)	0.5±0.0 (0.5)	2	2.0±1.1 (0.5-3.5)	1.4±0.6 (0.5-2.3)
*C. olivaceum** Cooke & Ellis	1	1.5±0.0 (1.5)	--	--	--	--	--	--	--
*C. spinosum** Chivers	1	4.0±0.0 (4.0)	--	--	--	--	--	--	--
*Cladosporium cladosporioides** (Fr.) De Vries	8	8.7±0.9 (1.0-24.0)	5.9±0.6 (0.5-20.0	8	6.4±0.8 (0.5-16.5)	4.9±0.7 (0.5-13.0)	8	11.4±1.2 (2.5-27.8)	10.1±0.9 (2.0-21.5)
Cladosporium spp.	1	16.0±0.0 (16.0)	14.0±0.0 (14.0)	1	18.0±0.0 (18.0)	10.0±0.0 (10.0)		18.0±0.0 (18.0)	23.5±0.0 (23.5)
*Curvularia lunata** (Wakker) Boedijn	6	1.3±0.2 (0.5-2.5)	1.4±0.2 (0.5-3.0)	2	0.9±0.3 (0.5-1.3)	--	7	2.8±0.2 (2.0-4.0)	4.9±0.3 (3.0-8.0)
*Doratomyces stemonitis** (Pers. extr.) Morton & Smith	1	3.5±0.0 (3.5)	--	--	--	--	--	--	--
*Drechslera australiensis** (Bugn) Subram & Jain ex M.B. Ellis	2	0.9±0.3 (0.5-1.3)	0.5±0.0 (0.5)	--	--	--	--	--	--
*D. hawaliensis** (Bugn) Subram & Jain	3	1.2±0.3 (0.5)	0.7±0.1 (0.5-1.0)	--	--	--	--	--	--
D. state of *Cochliobolus** *spicifer* Nelson	8	2.4±0.4 (0.5-6.0)	3.2±0.5 (1.0-7.0)	7	1.6±0.1 (0.5-2.5)	2.2±0.1 (0.5-3.5)	9	2.8±0.4 (2.0-4.3)	4.8±0.3 (3.0-10.5)
*Fusarium moniliforme** Sheldon	12	11.6±1.2 (2.5-25.0)	12.1±1.3 (3.0-27.5)	12	12.4±1.1 (4.0-27.0)	13.2±0.9 (3.5-36.3)	10	4.8±0.3 (2.0-10.5)	5.3±0.3 (2.5-16.0)
*F. subglutinous** (Wollenn & Rimking) Nelson, Toussound & Marasas comb.nov.	4	6.3±0.4 (5.0-10.5)	3.1±0.2 (1.5-15.3)	4	5.7±1.1 (2.8-12.0)	6.3±1.2 (2.5-18.5)	3	2.9±0.5 (1.0-5.0)	4.6±0.4 (2.5-6.3)
*F. oxysporum** Schlecht emend. Snyd. x Hans.	8	5.1±0.7 (1.0-16.0)	10.3±1.1 (2.0-22.5)	8	11.2±0.9 (3.0-24.5)	12.2±1.3 (3.3-29.3)	5	3.9±0.7 (0.5-9.0)	4.4±0.8 (0.5-13.0)
F. semitectum Berk x Rav.	4	2.9±0.5 (1.0-5.0)	4.6±0.4 (2.5-6.3)	4	5.7±1.1 (2.5-12.0)	6.3±0.4 (5.0-8.0)	4	2.4±0.4 (0.5-4.0)	2.1±0.4 (0.5-3.5)
F. solani (Mart.) Appel x Wollenw. emend. Snyd.Hans.	1	--	1.0±0.0 (1.0)	1	5.0±0.0 (5.0)	7.3±0.0 (7.3)	--	--	--
Macrophomina phaseolina	5	13.6±5.3	14.8±6.1	4	12.1±4.3	13.2±5.4	5	4.5±0.7	7.2±0.8

(cont'd....)

(Table I cont'd...)

(Tassi) Goid		(0.5-64.0)	(1.0-70.0)		(6.5-35.8)	(1.0-46.0)		(1.5-12.5)	(0.5-20.8)
*Memnomella echinata**	2	1.0-0.4	--	--	--	--	--	--	--
(Riv.) Gallowany		(0.5-1.5)							
Myrothecium roridum	6	1.4±0.2	1.8±0.3	7	1.3±0.2	2.7±0.3	1	0.5±0.0	1.0±0.0
Tode extr.		(0.5-3.0)	(0.5-4.5)		(0.5-4.0)	(1.0-4.5)		(0.5)	(1.0)
*Nigrospora oryzae**	2	0.5±0.0	0.6±0.3	2	0.9±0.3	0.5±0.0	2	--	0.5±0.2
(Berk x Br.) Petch		(0.5)	(0.3-1.0)		(0.5-1.3)	(0.5)			(0.2-0.8)
Penicillium spp.	1	4.0±0.0	--	1	4.3±0.0	1.0±0.0	1	13.0±0.0	6.3±0.0
		(4.0)			(4.3)	(1.0)		(13.0)	(6.3)
Rhizopus spp.	4	12.3±4.8	6.3±0.4	4	13.4±5.5	4.6±0.4	5	20.8±7.9	5.6±0.7
		(6.8-41.0)	(5.0-8.0)		(1.0-46.3)	(2.5-6.3)		(2.0-68.0)	(1.0-13.5)
*Scopulariopsis brumpti**	1	1.3±0.0	2.0±0.0	2	0.5±0.0	0.6±0.1	--	--	--
Salvanet-Duval		(1.3)	(2.0)		(0.5)	(0.3-1.0)			
Sporotrichum	2	4.0±0.0	1.25±0.0	1	--	1.0±0.0	--	--	--
*prunisporium**		(4.0)	(1.25)			(1.0)			
*Stachybotrys atra**	3	1.8±0.9	2.3±10.7	3	1.2±0.3	1.6±0.6	--	--	--
Corde		(0.5-3.0)	(0.5-4.5)		(0.5-2.0)	(0.5-3.5)			
*Trichurus spirilis**	1	2.0±0.0	--	--	--	--	--	--	--
Hasselring									

Data shows percentage of infected seeds ± standard error. SI = No. of samples infected.
Numbers in parenthesis indicate infection range. * New records of fungi associated with cucumber seeds.

findings of Limonard (1968), Khan *et al* (1988), Sundaras and Herimath (1978).

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