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EFFICACY OF THREE DETECTION TECHNIQUES FOR THE ASSESSMENT OF SEED MYCOFLORA OF CARROT IN SINDH

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Abstract

Using blotter, deep freezing and agar plate method of ISTA, the seed borne mycoflora of 5 carrot cultivars from Sind were examined. The pathogen isolated were *Alternaria alternata, Cladosporium cladosporioides, Fusarium moniliforme, F. oxysporum, F. semitectum, Macrophomina phaseolina, Penicillium* sp., and *Aspergillus candidus* whereas *Aspergillus flavus, A. glaucus, A. sulphureus, A. terreus, A. wentii, Chaetomium globosum, Chaetomium* sp., *Memnoniella echinata, Rhizopus nigricans, Stachybotryts atra, Ulocladium atrum* were non-pathogenic. Of these *Aspergillus flavus, Chaetomium globosum, Macrophomina phaseolina, Fusarium oxysporum,* and *F. monoliforme* were preponderant. Carrot cultivars obtained from Sukkur showed highest incidence of pathogenic fungi as compared to cultivar from Karachi and Tandojam.

Introduction

Carrot an important winter vegetable crop is attacked by various fungal pathogens like *Cercospora carotae* Pass, *Alternaria dauci* Khan, *Alternaria radicina* Meir, Drechsler & Eddy (Chupp & Sherf, 1960). In Pakistan, leaf blight (*Cercospora carotae* Pass), root rot (*Alternaria radicina* Meir, Drechler & Eddy), leaf spot (*Alternaria tenuissima* (Kunz ex Pers. Wiltshire), charcoal rot (*Macrophomina phaseolina* (Tassi.) Goid.) are seed borne (Mirza & Qureshi, 1978; Ghafoor & Khan, 1976). Only blotter technique has been used to study the seed borne mycoflora of carrot in Pakistan (Wahid *et al.*, 1988). The present study describes the efficacy of different techniques in the detection of pathogenic fungal species for distribution of disease free seed samples to the growers.

Materials and Methods

Cultural Techniques: Five seed samples of different carrot cultivars were collected from the farms and markets of Sind during 1987-88. Blotter, deep freezing and agar plate techniques as recommended by ISTA (Anon., 1976) were used.

a) In standard blotter method, 400 seeds from each samples were distributed on three layer of well soaked filter paper in sterilized 9 cm glass Petri dishes, 25 seeds per dish. Each cultivar was replicated 16 times. The dishes were incubated for 7 days at 25°C (± 1°C) under 12 h alternating cycles of artificial day light (ADL) from white fluorescent tube. The seeds were examined under stereomicroscope. Fungi encountered were identi-

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fied up to the species level using descriptions given by Ellis (1971); Booth (1971); Barnett & Hunter (1972); Nelson (1983) and Raper & Fennel (1965). In a comparable set, seeds were disinfected with 2% NaOCl for 2-3 minutes before placing the seeds on blotter papers.

b) In deep freezing method, seeds of each sample were surface sterilized for three minutes in 2% NaOCl and placed without washing on blotter paper as described above. The Petri dishes after initial incubation for 24 h at 25°C, were kept in freezer for 48 h, and then re-incubated at 25°C (± 1°C) under 12 h alternating cycles of artificial day light and darkness. The plates were examined after 7 days.

c) In agar plate method, also known as Ulster method (Muskett & Malone, 1941), 400 seeds from each sample surface disinfected with 1% NaOCl for 3 minutes and washed in sterile distilled water were placed on Potato Dextrose Agar 25 seeds per plate. The plates were incubated for 7 days at 24°C (±1°C) in alternating cycles of light and darkness.

Statistical Methods

a) Test of significance of differences in the proportion of seeds infected by pathogenic and non-pathogenic fungi (cultivar and cultural methods) were evaluated for each of the treatments separately using the Z-test (Zar, 1974).

(b) *Species Diversity:* Species richness was expressed as the total number of species recorded (S). General diversity which incorporates the species richness and equitability, components of diversity was evaluated by using Margalef's (1957) $\overline{H} = -\sum_{i=1}^{S} p_i \log p_i$ and Simpson's (1949) index $\lambda = \sum_{i=1}^{S} \left[\frac{n_i (n_i-1)}{[N (N-1)]}\right]$ where p*i* is the proportion of seeds infected by a species, n_i is the number of seeds infected by a species, S equals the total number of species and N equals the total seed infected. Equitability, expressing the evenness of allotment of individual incidences of seed infection by various fungal species was ascertained using the index(e) proposed by Pielou (1969) $e = \overline{H}/\overline{H}$ max where $\overline{H}$ max = log S.)

(c) *Multivariate Analysis:* The existence of group structure, if any, was evaluated using unweighted pair group average technique of cluster analysis using Euclidean distance as the resemblance function (Ludwig & Reynold, 1988).

Results and Discussion

Of the 19 fungal species isolated from seeds of 5 carrot cultivars using ISTA techniques (Anon., 1976), *Alternaria alternata, Aspergillus candidus, Cladosporium cladosporioides, Fusarium moniliforme, F. oxysporum, F. semitectum, Macrophomina phaseolina,* and *Penicillium* sp., were pathogenic (Chupp & Sherf, 1960; Mirza & Qureshi, 1978), in which *F. moniliforme, F. oxysporum, F. semitectum, M. phaseolina* and *A. alternata* were preponderant and encountered in the three cultural methods used. The incidence percentage varied with the cultivars. The non-pathogenic isolates included

Table 1. Number of Fungi recorded from 5 carrot cultivars.

Fungi	P/NP	Blotter Method					Deep Freezing Method					Agar Plate Method				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alternaria alternata	P	–	80	–	–	-	48	44	32	28	52	20	44	44	40	32
Aspergillus candidus	P	8	–	–	12	–	–	–	–	–	–	4	12	–	12	12
A. flavus	NP	32	8	12	148	36	36	28	32	40	52	80	80	44	44	36
A. glaucus	NP	–	–	–	–	–	–	–	–	–	1	–	1	–	–	–
A. sulphureus	NP	16	–	–	–	16	–	–	16	–	–	–	–	–	–	–
A. terreus	NP	8	–	–	–	12	–	–	–	20	16	–	–	12	–	8
A. wentii	NP	16	–	12	24	–	–	–	–	–	–	–	–	–	–	-
Chaetomium globosum	NP	28	40	32	32	32	56	40	36	44	32	12	20	20	16	32
Chaetomium spp.	NP	–	–	–	–	–	52	40	32	–	20	–	–	–	–	–
Cladosporium cladosporioides	P	20	24	24	–	20	–	–	–	–	–	–	–	–	–	–
Fusarium moniliforme	P	–	–	80	104	88	–	–	60	44	48	–	–	56	48	64
F. oxysporum	P	28	64	28	36	88	32	–	–	40	72	24	20	–	56	80
P. semitectum	P	–	8	–	–	8	–	28	–	–	–	20	24	80	52	–
Macrophomina phaseolina	P	132	–	32	36	28	56	48	48	44	32	36	40	40	36	32
Memnoniella echinata	NP	4	–	8	–	8	8	12	–	8	–	–	–	–	–	–
Penicillium sp.	P	–	–	4	–	–	–	–	–	–	–	–	–	–	–	–
Rhizopus nigricans	NP	–	–	–	–	–	–	–	–	–	–	16	28	48	–	–
Stachybotryts atra	NP	–	12	–	–	–	–	–	–	–	–	–	–	–	–	–
Ulocladium atrum	NP	–	8	–	–	–	4	4	8	8	8	–	–	–	–	–

P = Pathogenic; NP = Non-Pathogenic; 1 = Karachi; 2 = Tandojam; 3 = Mirpurkhas; 4 = Hyderabad; 5 = Sukkur.

Table 2. Test of significance (z-values) of differences in the proportion of seeds of various cultivars infected by pathogenic fungi under the three different cultural methods.

BM	2	.852													
	3	1.712	.858												
	4	.283	1.135	1.996											
	5	3.134	4	4.879	2.847										
DFM	1	3.778	2.914	2.050	4.066	7.016									
	2	5.017	4.144	3.272	5.310	8.314	1.213								
	3	3.476	2.614	1.751	3.764	6.701	.297	1.511							
	4	2.292	1.436	.577	2.578	5.476	1.470	2.689	1.172						
	5	1.132	1.987	2.851	.848	1.992	4.936	6.193	4.631	3.436					
APM	1	6.320	5.434	4.552	6.618	9.692	2.478	1.261	2.777	3.963	7.518				
	2	5.336	4.460	3.586	5.630	8.650	1.524	.310	1.822	3.002	6.517	.950			
	3	2.270	3.130	4.002	1.985	.856	6.113	7.392	5.803	4.593	1.134	8.744	7.722		
	4	4.012	4.885	5.774	3.723	.864	7.924	9.264	7.622	6.378	2.863	10.67	9.606	1.722	
	5	2.270	3.130	4.002	1.985	.856	6.113	7.392	5.803	4.593	1.134	8.744	7.772	0	1.722
		1	2	3	4	5	1	2	3	4	5	1	2	3	4
		BM					DFM					APM			

1. Karachi, 2. Tando Jam, 3. Mirpur Khas, 4. Hyderabad, 5. Sukkur.

Aspergillus flavus, A. glaucus, A. sulphureus, A. terreus, A. wentii, Chaetomium globosum, Chaetomium sp., *Memnoniella echinata, Rhizopus nigricans, Stachybotrys atra,* and *Ulocladium atrum* in which *A. flavus* and *C. globosum* were frequently isolated (Table 1). Higher number of fungi (17 species) were recorded in blotter method followed by deep freezing (13 species) and agar plate method (11 species). *Alternaria radicina, Botryts cinerea, Fusarium solani,* and *Stemphyllium botryosum* reported by Wahid (1988) were not encountered in the present study.

The results of the test of significance of differences in the proportion of seed infected by pathogenic fungi in 5 cultivars under different cultural conditions are given in Table 2. Cultivar from Sukkur (CV-5) showed consistently higher percentage of pathogenic fungi compared to the other 4 cultivars in the test methods used ($p \leq 0.05$). Cultivar from Hyderabad (CV-4) also exhibited significantly higher incidence of pathogenic fungi than other cultivars (except CV-5) particularly in the agar plate method ($p \leq 0.05$). The cultivars from Karachi (CV-1) and Tandojam (CV-2) were found less infected with the pathogenic fungi compared to other 3 cultivars, particularly in the blotter and agar plate method ($p \leq 0.05$, Table 2). The significance of differences in the proportion of seeds infected by non-pathogenic fungi in various cultivars under different test procedures shows significantly higher incidence for cultivars from Karachi and Sukkur than other cultivars in the blotter method ($p \leq 0.05$ Table 3). The cultivars from Tandojam (CV-2) and Mirpurkhas (CV-3) yielded significantly lower incidence of non-pathogens compared to other cultivars ($p < 0.01$ or $p < 0.001$) in the deep freezing method. Species richness varied in the order: blotter method > deep freezing method > agar plate method. General diversity (H) and equitability (e) were found to vary in the order: deep freezing > agar plate method > blotter method (Table 4).

In the dendrogram (Fig. 1), 2 main groups (A and B) can be seen which represent the cultivars less infected by pathogens (group A) and those with higher incidence of pathogenic fungi (group B). Group A includes cultivars from Karachi and Tandojam, while group B includes cultivars from Hyderabad and Sukkur. The cultivar from Mirpurkhas is intermediate showing low incidence of pathogens under deep freezing method and higher incidence in blotter and agar plate method. The dendrogram resulting from the cluster analysis of cultivars and cultural methods with respect to % incidence of non-pathogenic fungi in seeds (Fig. 2) also shows 3 distinct groups (A, B and C). The large group (A) clusters together the cultivars under different cultural methods yielding higher incidence % of non-pathogenic fungi. Group B includes 3 cultivars showing higher incidence of non-pathogens in agar plate method while group C includes 3 cultivars showing lower incidence in the deep freezing test procedure.

It would appear that the 3 isolation techniques differ in their capability to detect specific pathogenic and non-pathogenic fungi associated with the seeds of different cultivars of carrot. Blotter method was generally more successful in the detection of pathogenic fungi such as *Cladosporium cladosporioides, F. moniliforme, F. oxysporum, F. semitectum, M. phaseolina,* and *Penicillium* sp., whereas, *A. alternata* was frequently detected in deep freezing and agar plate methods.

Table 3. Test of significance (z-values) of differences in the proportion of seeds of various cultivars infected by non-pathogenic fungi under the three different cultural methods.

BM	2	3.116													
	3	1.568	4.699												
	4	3.116	0	4.699											
	5	2.560	.556	4.139	.555										
DFM	1	3.963	7.147	2.380	7.147	6.576									
	2	1.568	4.699	0	4.699	4.139	2.380								
	3	1.568	4.699	0	4.699	4.139	2.380	0							
	4	.950	4.074	.617	4.074	3.516	3.002	.617	.617						
	5	2.177	5.317	.606	5.317	4.755	1.771	.606	.606	1.224					
APM	1	.320	3.439	1.247	3.439	2.882	3.638	1.247	1.247	.630	1.855				
	2	1.874	5.009	.304	5.009	4.449	2.074	.304	.304	.921	.301	1.552			
	3	1.568	4.699	0	4.699	4.139	2.380	0	0	.617	.606	1.247	.304		
	4	3.889	.771	5.476	.771	1.326	7.940	5.476	5.476	4.849	6.097	4.212	5.787-	5.476	
	5	2.379	.736	3.957	.736	.181	6.390	3.975	3.957	3.334	4.572	2.700	4.265	3.957	1.508
		1	2	3	4	5	1	2	3	4	5	1	2	3	4
		BM					DFM					APM			

1. Karachi, 2. Tando Jam, 3. Mirpur Khas, 4. Hyderabad, 5. Sukkur.

Table 4. Species richness(s) diversity (H and λ) equitability (e) of the fungal species and their percentage occurrence (abundance) recorded in various cultivars and cultural methods.

		Measures of Diversity + Equitability			
Cultural Methods	Cultivar	S	H	λ	e
Blotter Method	1	10	1.808635	.2445511	.7854803
	2	8	1.72551	.2152736	.8297949
	3	9	1.896036	.1855501	.8629233
	4	7	1.640616	.2392087	.8431098
	5	10	1.982024	.1704335	.8607821
Deep Freezing Method	1	8	1.895204	.1575578	.9114003
	2	8	1.934054	.1505093	.9300834
	3	8	1.961583	.1487499	.9433221
	4	9	2.065651	.132332	.9401186
	5	9	2.047949	.1395188	.932062
Agar Plate Method	1	8	1.793393	.2073683	.8624399
	2	8	1.919924	.1673095	.9232883
	3	8	1.968267	.1484169	.9465364
	4	8	1.986934	.1419142	.9555132
	5	8	1.890014	.1692167	.9089044

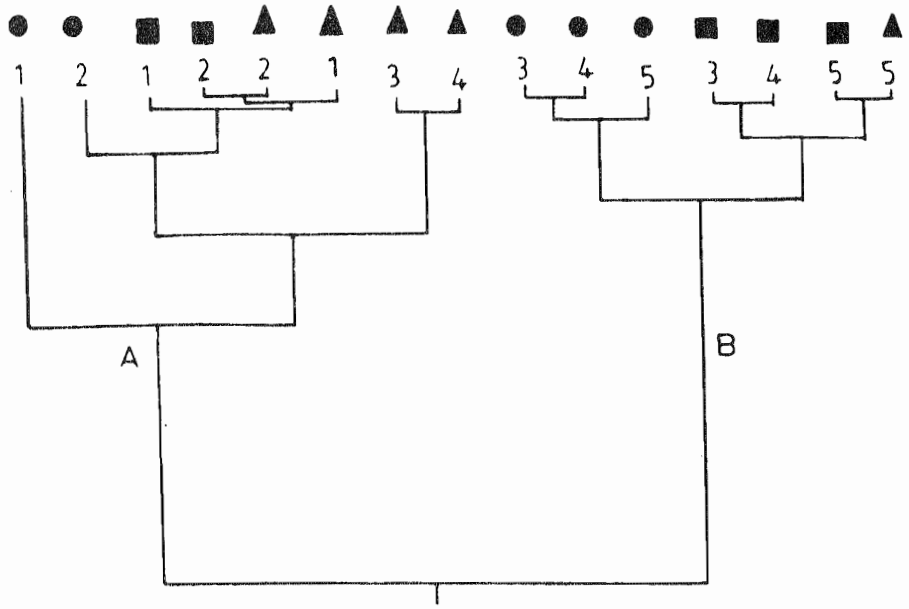

Fig. 1. Dendrogram resulting from cluster analysis of 5 cultivars and 3 culture methods based on the records of percentage incidence of seed infection by pathogenic fungi ●BM ▲DFM ■APM.

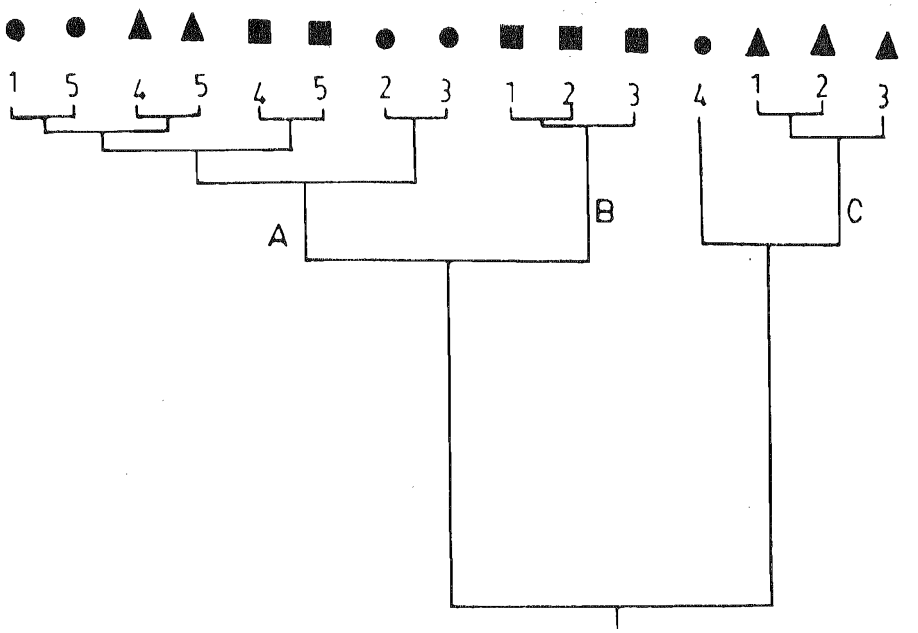

Fig. 2. Dendrogram resulting from cluster analysis of 5 cultivars and 3 culture methods based on the records of percentage incidence of seed infection by non-pathogenic fungi ●BM ▲DFM ■APM.

Khan *et al.*, (1988) found blotter method more effective in the detection of general mycoflora of rice seeds. In the present study agar plate method was most effective in the detection of non-pathogenic fungi including *Aspergillus* sp., *Chaetomium* spp., and *Rhizopus nigricans*, as also observed by Limonard (1968) and Tempe (1970).

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