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SEED-BORNE MYCOFLORA OF *CAPSICUM ANNUUM* IMPORTED FROM INDIA

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Abstract

Using standard blotter and deep-freezing techniques, seed-borne mycoflora of 40 samples from consignments of *Capsicum annuum* L. (red chillies var. Dhora, imported from India) was studied. Of the 47 fungal species *Absidia corymbifera*, *Acremonium fusidioides*, *Aspergillus tamarii*, *Blakeslea* sp., *Cephaliophora irregularis*, *Cladosporium accacicola*, *Scopulariopsis* sp., *Streptomyces* sp., *Tritirachium* sp., and *Ulocladium tuberculatum* have been not reported before from seeds as well as pericarp of *C. annuum*.

Introduction

Capsicum (*Capsicum annuum* L.) is an important crop and extensively cultivated throughout the tropics and Southern countries such as Bangladesh, India, Pakistan and Sri Lanka. Nutritionally, it contains significant amounts of vitamins A and C. The dried ripe chillies are used as condiment for culinary purposes and seasoning. In medicine, it is used as powerful stimulant and carminative and also to counter irritant. In the present study, mycoflora associated with seeds and fruits of *Capsicum annuum* imported from India has been examined and compared with seed-borne diseases (Khan *et al*., 1974a&b; Ghafoor & Khan, 1976; Mirza & Qureshi, 1978; Richardson, 1979,1981, 1983; Nasreen *et al*., 1988, 1992; Hashmi, 1989; Hashmi & Hasal, 1989; Mushtaq & Hashmi, 1997; Abbas *et al*., 2004).

Materials and Methods

Forty samples of *C. annuum* (red chillies var. Dhora) imported from India were collected from the Department of Plant Protection, Govt. of Pakistan, Karachi and intercepted at Plant Quarantine Office, Lahore. Seeds and pericarp were separately analyzed for the presence of fungi by standard blotter (Anon., 1976) and deep-freezing (Limonard, 1968) methods. Four hundred seeds and 75 pieces of pericarp for each sample were plated on 3 layered moistened blotter discs in 9 cm glass Petriplates @ 25 seeds and 15 pieces of pericarp per plate. Plates were incubated for 7 days at 22±1°C in Eyela La 1000 low temperature incubator. Incubated seeds were examined under microscope. In deep-freezing method, seeds and pericarp were incubated at 22±1°C for 24 hrs., followed by incubation at -20±1°C for 24 hrs., and then at 22±1°C for 5 days. In both methods growing fungi were isolated and purified on potato dextrose agar (PDA), corn meal agar (CMA), and speziellier nahrstoffarmer agar (SNA). The fungi isolated were identified after reference to Barnet & Hunter (1972), Booth (1971), Domsch *et al*., (1980), Joffe (1986), Neergaard (1977), Nelson *et al*., (1983), Nirenberg (1990), Pascoe (1990a,b), Raper & Fennel (1965) and Singh *et al*., (1991). Data of seed-borne mycoflora and pericarp was statistically analyzed by using computer-based software SPSS version 10.

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Results and Discussion

A total number of 47 species of fungi belonging to 30 genera by standard blotter method as compared to 16 genera and 23 species by deep-freezing method were isolated from seed samples of *Capsicum annuum* (Table 1). Similarly from pericarp, 32 species belonging to 23 genera by standard blotter method and 17 species belonging to 14 genera were isolated by deep-freezing method (Table 2). The isolated fungi were identified as: *Absidia corymbifera*, *Acremonium fusidioides*, *Acremonium* sp., *Alternaria alternata*, *Aspergillus candidus*, *A. flavus*, *A. niger*, *A. ochraceus*, *A. sulphureus*, *A. tamarii*, *A. terreus*, *A. versicolor*, *Aspergillus* spp., *Bipolaris australiensis*, *B. spicifera*, *Blakeslea* sp., *Cephaliophora irregularis*, *Cephalosporium* sp., *Chaetomium bostrychodes*, *C. globosum*, *Cladobotryum varium*, *Cladosporium accacicola*, *Cladosporium* spp., *Colletotrichum capsici*, *Curvularia lunata*, *C. pallescens*, *Cylindrocarpon* sp., *Drechslera* spp., *Exserohilum holmii*, *E. rostratum*, *Emericella* sp., *Epicoccum* sp., *Fusarium chlamydosporum*, *F. moniliforme*, *F. pallidoroseum*, *F. proliferatum*, *F. solani*, *F. sporotrichioides*, *F. subglutinans*, *Macrophomina phaseolina*, *Memnoniella echinata*, *Myrothecium* sp., *Paecilomyces* sp., *Penicillium* spp., *Phoma* sp., *Rhizoctonia solani*, *Rhizopus stolonifer*, *Scopulariopsis* sp., *Stachybotrys atra*, *Streptomyces* sp., *Trichoderma harzianum*, *Tritirachium* sp., *Ulocladium tuberculatum* and *Verticillium albo-atrum*.

Aspergillus ochraceus, *Bipolaris australiensis*, *Chaetomium bostrychodes*, *Fusarium chlamydosporum*, *F. sporotrichioides* and *Ulocladium tuberculatum* appear to be new reports only from seeds, whereas, *Aremonium fusidioides*, *Acremonium* sp., *Aspergillus terreus*, *Cladobotryum varium*, *Cladosporum acacicola* and *Emericella* sp., from pericarp of *C. annuum*. On the other hand *Absidia corymbifera* sp., *Aspergillus tamarii*, *Blakeslea* sp., *Cephaliophora irregularis*, *Scopulariopsis* sp., *Streptomyces* sp., and *Tritirachium* sp., have been isolated for the first time from seeds as well as pericarp of *C. annuum*. It may be mentioned that species of *Alternaria*, *Colletotrichum*, *Fusarium* and *Phoma* have been reported by Hashmi (1990) from samples of capsicum imported from India.

Significant differences were found between occurrence of fungi in blotter and deep-freezing methods by Bonferroni test that was further confirmed by ANOVA (Table 3 & 4). Occurrence of fungal species was more frequent in blotter test as compared to deep-freezing in both seeds and pericarps. However, for the detection of slow growing and parasitic fungi deep-freezing method appeared more suitable, where the dead embryo of seed provides nourishment, moreover, growth of fast growing saprophytic fungi is checked due to an interrupting deep-freezing period of 24 hrs. Fast growing saprophytic fungi like, *Aspergillus* spp., *Cladosporium* spp., *Penicillium* and *Rhizopus* spp., may be troublesome particularly in the detection of slow growing parasites present internally (Tempe, 1970). *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Chaetomium bostrychodes*, *Drechslera tetramera*, *Fusarium moniliforme*, *F. pallidoroseum*, *Paecilomyces* sp., and *Rhizopus stolonifer* were predominantly isolated from seeds of capsicum by both blotter and deep-freezing methods. Likewise, *Absidia corymbifera*, *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Colletotrichum capsici*, *Fusarium pallidoroseum*, *F. solani*, *Rhizopus stolonifer*, *Streptomyces* sp., and *Verticillium albo-atrum* were predominantly isolated from pericarp.

Table 3. Analysis of variance of major seed-borne fungi of *Capsicum annuum*.

Source	Sum of squares	df	Mean square	F	Probability
Methods (A)	306.9	1	306.9	1052.867	0.001
Fungi (B)	1643.678	9	182.631	627.732	0.001
Samples (C)	946.131	19	49.796	171.158	0.001
A*B	775.865	9	86.207	296.309	0.001
A*C	892.206	19	46.958	161.403	0.001
B*C	4977.553	171	29.108	100.051	0.001
A*B*C	5329.716	171	31.168	107.129	0.001
Error	**116.375**	**400**	**0.291**		

Table 4. Analysis of variance of major fungi isolated from pericarp of *Capsicum annuum*.

Source	Sum of squares	df	Mean square	F	Probability
Methods (A)	315.451	1	283.378	976.139	0.001
Fungi (B)	1452.301	10	178.537	595.396	0.001
Samples (C)	112.326	15	38.387	155.138	0.001
A*B	678.112	10	76.105	269.893	0.001
A*C	732.415	15	38.283	127.111	0.001
B*C	3849.678	150	23.819	89.013	0.001
A*B*C	5115.392	150	27.137	98.153	0.001
Error	**96.497**	**400**	**116**		

Quality of seeds and fruits of capsicum were destroyed due to infestation of these fungi that produce mycotoxins causing health hazards in human beings and animals (Hiscocks, 1965). Hashmi (1990) reported anhydrofusarubin, bostrycoidin, fusarubin, moniliformin, vomitoxin (deoxynivalenol), zearalenol and zeraralenone mycotoxins from *F. equiseti*, *F. moniliforme*, *F. oxysporum*, *F. pallidoroseum* and *F. solani* isolated from *Capsicum annuum*. These mycotoxins are hazardous to human and animal health and mostly carcinogens, mutagens and teratogens (Marasas *et al*., 1984). Of the various fungi isolated from seeds and pericarp of capsicum, *Aspergillus flavus* was most predominant, known to produce aflatoxin B1, B2, G1 and G2 in food and feed stuff, whereas, *A. ochraceus* produces ochratoxins (Pohland & Wood, 1987). Similarly species of *Alternaria* produce large number of secondary metabolites (idiolites) but most of them have not been evaluated toxicologically. Considering the occurrence of pathogenic and toxigenic fungal species it is proposed that the import of red chillies must be prohibited to prevent entrance of exotic fungi in country as well as to prevent health hazard in national interest.

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