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SEED - BORNE MYCOFLORA OF *CAPSICUM ANNUUM* L.

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Abstract

Seed - borne mycoflora of capsicum from 30 countries was examined. Seed samples from Pakistan, Turkey, India, Nigeria and Ghana showed maximum infection of fungi viz., *Alternaria alternata, Colletotrichum capsici, Fusarium moniliforme, F. semitectum, F. solani, F. equiseti, F. oxysporum, Phoma capsici, Verticillium albo-atrum* and *Myrothecium roridum* alongwith certain unidentified yeast and bacteria. Of these, *F. moniliforme* and *A. alternata* were predominant. Other fungi isolated were *Alternaria solani, Ascochyta capsici, Botrytis cinerea, Colletotrichum gloeosporioides, Cercospora capsici. Macrophomina phaseolina* and *Myrothecium verrucaria*. In pathogenicity tests *F. moniliforme, F. solani* and *F. oxysporum* caused seed rot and wilting of capsicum seedlings.

Introduction

Capsicum *(Capsicum annuum* L.*)* is extensively cultivated throughout the tropics and southern European countries. Nutritionally, it contains significant amounts of vitamins A and C. The dried ripe fruit is used for culinary purposes and for seasoning. In medicine, it is internally used as powerful stimulant and carminative, and externally as a counter irritant. Of the various disease causing organisms the seed-borne mycoflora of capsicum is described in the Annotated List of Seed-borne Diseases (Richardson, 1979, 1981, 1983) . From the extensive collection of seed samples obtained from 30 different countries of the world the seed-borne mycoflora was studied. This is presented in this paper. The pathogenicity of *F. moniliforme*, *F. solani*, and *F. oxysporum* on capsicum was also investigated.

Materials and Methods

Two hundred twenty-two seed samples of capsicum obtained from 30 countries through the courtesy of the Danish Government Institute of Seed Pathology, Copenhagen, Denmark were used in this study. The standard blotter method for seed testing was used (Anon., 1966). Pathogenicity tests were conducted according to the techniques described by Hashmi (1988). Pure cultures of fungi were maintained on potato dextrose agar (pH5.6) and identified according to Nelson *et al.*, (1983), Ellis (1971) and Barron (1968).

Table 1. Fungi isolated from seeds of *Capsicum annuum* obtained from seeds of different countries of the world.

Countries	Number of samples tested	*Alternaria alternata* A	*Alternaria alternata* B	*Colletotrichum capsici* A	*Colletotrichum capsici* B	*Fusarium equiseti* A	*Fusarium equiseti* B	*Fusarium moniliforme* A	*Fusarium moniliforme* B	*Fusarium semitectum* A	*Fusarium semitectum* B	*Fusarium solani* A	*Fusarium solani* B	*Phoma capsici* A	*Phoma capsici* B
Algeria	2			1			1.00±0.00 (1.00)	2	3.50±0.35 (3.00-4.00)	1	1.00±0.00 (1.00)			1	1.00±0.00 (1.00)
Argentina	5			1	1.50±0.00 (1.50)	1	2.50±0.00 (2.50)	1	4.00±0.00 (4.00)	2	11.50±10.50 (1.00-22.00)	1	1.00±0.00 (1.00)	3	3.66±1.45 (1.00-6.00)
Brazil	1			1	4.00±0.00 (4.00)			1	10.00±0.00 (10.00)						
Burma	1	1	1.00±0.00 (1.00)					1	24.00±0.00 (24.00)						
China	5			3	5.00±1.80 (1.00-8.00)			4	1.75±0.24 (1.00-3.00)	1	1.00±0.00 (1.00)	1	1.00±0.00 (1.00)	2	1.50±0.35 (1.00-2.00)
Cuba	3							2	2.00±0.00 (2.00)					3	12.00±7.00 (2.00-28.00)
Egypt	2	2	8.50±3.18 (4.00-13.00)	1	2.00±0.00 (2.00)	1	1.00±0.0 (1.00)	2	4.50±0.35 (4.00-5.00)	1	4.00±0.00 (4.00				
								1	1.00±0.00 (1.00)						
Ghana	6	1	11.00±0.00 (11.00)	5	3.10±1.55 (1.00-8.50)	1	1.00±0.00 (1.00)	4	12.62±3.15 (1.00-35.00)						
India	50	36	8.75±2.83 (8.00-21.00)	33	9.68±7.25 (1.60-63.00)	16	10.87±6.10 (1.00-100.00)	31	7.32±2.71 (1.00-69.00)	19	10.95±1.58 (1.00-41.00)	9	1.00±0.00 (1.00)	21	4.02±1.12 (1.00-53.00)
Indonesia	8			7	4.85±1.00 (3.00-7.50)	1	1.00±0.00 (1.00)	5	2.70±0.52 (1.00-6.00)	4	20.62±3.75 (2.00-65.50)	4	1.87±0.14 (1.00-4.00)	4	3.25±0.17 (1.00-5.00)
Jamaica	2					1	1.00±0.00 (1.00)	2	2.75±0.53 (2.00-3.50)			1	23.00±0.00 (23.00)	1	4.00±0.00 (4.00)
Korea	5	5	8.60±2.20 (3.00-14.00)			2	17.50±11.66 (1.00-34.00)	5	13.220±6.82 (1.00-62.00)	1	31.00±0.00 (31.00)			1	2.00±0.00 (2.00)
Malaysia	2	2	2.00±0.00 (2.00)		2.00±0.00 (2.00)			1	3.00±0.00 (3.00)					1	1.00±0.00 (1.00)

Mauritius	7	7	14.94±4.00 (1.00-25.00)		2.00±0.00 (2.00)	1	1.50±0.00 (1.50)	7	19.78±17.82 (1.00-94.00)	2	13.00±3.53 (3.00-23.00)	1	2.50±0.00 (2.50)	7	2.14±0.31 (1.00-6.00)
Morocco	4	4	7.12±3.13 (7.00-16.00)	1	10.50±6.71 (1.00-20.00)			2	2.25±0.08 (2.00-2.50)	2	1.00±0.00 (1.00)	1	1.00±0.00 (1.00)	1	1.00±0.00 (1.00)
Nepal	2			1	7.00±0.00 (7.00)									1	1.00±0.00 (1.00)
Nigeria	8	5	5.52±1.82 (3.00-11.00)	3	6.52±6.90 (5.00-8.50)			4	47.12±8.70 (1.00-78.50)	3	17.66±2.80 (4.50-36.50)	2	6.00±0.36 (4.00-8.00)	4	3.75±0.55 (1.00-12.00
Pakistan	13	12	8.01±2.69 (1.00-19.00)	7	10.00±7.80 (1.00-43.00)	4	1.25±0.12 (1.00-2.00)	10	11.65±4.19 (1.00-83.00)	3	7.50±1.35 (1.00-2.00)	2	1.00±0.00 (1.00)	6	1.83±0.08 (1.00-3.50)
Peru	3	1	2.50±0.00 (2.50)			2	1.50±0.35 (1.00-2.00)			2	1.50±0.17 (1.00-2.00)	1	1.00±0.00 (1.00)	1	2.00±0.00 (2.00)
Philippines	4	1	2.00±0.00 (2.00)	1	1.50±0.00 (1.50)			4	11.12±4.57 (1.00-38.50)	1	1.00±0.00 (1.00)	1	1.00±0.00 (1.00)	3	8.33±1.82 (2.00-21.00)
Romania	2							2	(1.50±0.35 (1.00-2.00)			1	1.00±0.00 (1.00)	2	2.75±0.62 (1.00)-4.50)
Sri Lanka	4	4	3.25±1.01 (1.50-6.00)	2	6.00±3.53 (1.00-11.00)	2	2.75±0.44 (1.50-4.00)	2	1.50±0.11 (1.00-2.00)	1	4.00±0.00 (4.00)				
Sudan	2	2	3.50±0.00 (3.50)					2	1.50±0.35 (1.00-2.00)			2	1.00±0.00 (1.00)		
Suriname	1	1	7.50±0.00 (7.50)	1	1.00±0.00 (1.00)			1	11.00±0.00 (11.00)						
Thailand	4	1	3.00±0.00 (3.00)	3	43.83±7.39 (26.00-78.00)			3	11.33±2.17 (1.00-26.00)	1	10.00±0.00 (10.00)			2	1.50±0.35 (q.00-2.00)
Trinidad	1									1	8.00±0.00 (8.00)	1	1.00±0.00 (1.00)	1	38.00±0.00 (38.00)
Turkey	70	48	10.41±3.88 (1.00-35.00)	7	1.85±0.01 (1.00-1.00)	10	5.95±0.48 (1.00-20.50)	46	16.35±1.55 (1.00-98.00)	11	1.90±0.27 (1.00-4.00)	12	3.37±0.54 (1.00-12.50)	29	5.36±1.23 (1.00-40.00)
Vietnam	2	1	5.00±0.00 (5.00)	1	15.00±0.00 (15.00)			1	4.00±0.00 (4.00)			1	2.00±0.00 (2.00)	2	3.50±1.76 (1.00-6.00)
Zambia	1	1	8.00±0.00 (8.00)	1	4.0±0.00 (4.00)			1	2.50±0.0 (2.50)						
$\bar{X}$			6.33		7.08		3.68		8.77		8.98		1.80		1.00
Standard Error			1.85		0.78		0.30		1.56		0.40		0.01		0.00

A: No. of infected samples, B: Infection percentage., Numbers in parentheses indicate infection range.

Results and Discussion

Of the 222 samples of capsicum, 64.0% samples were infected by *Fusarium moniliforme* and 60.8% by *Alternaria alternata.* This was followed by *Phoma capsici,* 44.6%; *Colletotrichum capsici,* 36.9%; *F. semitectum,* 26.5%; *F. solani,* 20.2%; *F. equiseti,* 19.8%; *Verticillium albo-atrum,* 10.8%; *Myrothecium roridum,* 7.2%; *F. oxysporum,* 4.9%; *Macrophomina phaseolina,* 4.4%; *Botrytis cinerea,* 3.1%; *Myrothecium verrucaria,* 1.8%; *Alternaria solani,* 1.3% *Colletotrichum gloeosporioides,* 0.9%; *Cercospora capsici* , 0.9%; and *Ascochyta capsici* , 0.9% (Table 1).

It is interesting to note that *M.. roridum* (Av. 9.47 ± 0.27) was the most prevalent fungus observed on capsicum samples of some Asian and African countries as well as of Romania and Turkey. *F. semitectum* (Av.8.98 ± 0.40) was predominant in samples from Argentina, Indonesia, India and Jamaica. *F. moniliforme* (Av. 8.77 ± 0 55) was predominant in samples of Pakistan, Turkey and India. The anthracnose fungus, *Colletotrichum capsici* (Av. 7.08 ± 0.78) was abundantly represented in samples of Thailand, Pakistan and India. *C. capsici,* however, was less prevalent in samples of African and Latin American countries. *Verticillium albo-atrum* (Av.6.87 ± 5.37) was recorded in large amounts from samples of India and Turkey. *Alternaria alternata* was prevalent mainly in samples of India, Pakistan, Mauritius, Morocco and Turkey. This was followed by *F.equiseti* (Av.3.68 ± 0.30) and *F. oxysporum* (Av.3.31 ± 0.41). While *F. equiseti* appeared more frequently in samples of India, Turkey and Korea, *F. oxysporum* was prevalent in samples of Turkey and Indonesia only. Other seed-borne fungi occurring in traces as a chance associate of capsicum seed were *Colletotrichum gloeosporioides* (Av. 2.00+ 0.70) , *Botrytis cinerea* (Av.1.20 ± 0.05), *Macrophomina phaseolina* (Av.1.18 ± 0.00), *Myrothecium verrucaria* (Av.1.12 ± 0.00), *Alternaria solani* (Av.1.00 ± 0.00), *Ascochyta capsici* (Av.1.00 ± 0.00) and *Cercospora capsici* (Av.1.00 ± 0.00). *Phoma capsici,* although isolated from 44.6% samples, was present only in traces (Av. 1.00± 0.00). *F. moniliforme* caused 6.0 to 8.0% loss in germination of the seeds (Table 2). All ungerminated seeds were covered with growth of *F.moniliforme.* Mortality of the seedlings was 3.0 to 4.0% due to profuse growth of *F.moniliforme* on seed coat. The fungus was also found growing on seed coat of number of seedlings (13. 8 to 18. 0%) which looked apparently healthy. The seed coat of many healthy seedlings (56. 0 to 75.4%) did not show any visible growth of the fungus. It was surprising to note that when different parts of such seedlings were incubated on wet filter paper, *F. moniliforme* was isolated, 56. 0% from the roots, 17. 0% from the hypocotyl as compared to 7. 0% from the seed coat. Where seeds were sown in test tubes on cotton plugs with high humidity, *F. moniliforme* became more virulent and killed 34. 0% of the seedlings (Table 2).

Table 2. Effect of seed-borne infection of *Fusarium moniliforme* in capsicum.

Categories	Expt. 1	Expt. 2	Expt. 3
	Transplantation of 130 seedlings from blotter to pots	Direct sowing of 50 seeds in pots	Sowing of 50 seeds in test tubes on cotton plugs
	% seeds and seedlings in each category		
Healthy looking seedlings	75.5	56.0	—
Healthy looking seedlings with mycelial growth of *F. moniliforme* on seed coat	13.8	18.0	—
Healthy looking seedlings with profuse conidial growth of *F. moniliforme*	7.7	14.0	—
Seedlings killed, seed coat covered with *F. moniliforme*	3.0	4.0	34.0
Rotted seeds covered with *F. moniliforme*	—	8.0	6.0

F. solani caused 16. 0 to 36. 0% seed rot, all ungerminated seeds were heavily covered by fungal growth of *F. solani*. The germinated seedlings showed 24. 0 to 34. 0% wilting, where seedlings were inoculated by *F. solani*, 64. 0% mortality was observed (Table 3). Effect of necrosis and deformation of primary as well as secondary roots was observed in 20. 0 to 28. 0% of plants. Twelve to 36. 0% of healthy looking plants did not show any external growth of the fungus, however, *F. solani* was isolated in varying percentage from their components indicating a systemic nature and pathogenicity of the fungus.

Capsicum seeds, 8.2 to 41.2% rotted in sand and in test tubes (Table 3) where heavy growth of *F. oxysporum* was observed . *F. oxysporum* killed up to 36. 5% seedlings depending on different experimental conditions. Thirty-one to 38. 0% healthy looking seedlings in sand as well as 35. 0% in test tubes were devoid of any external growth of the pathogen. However, these seedlings on component plating yielded *F. oxysporum* in varying percentage. In pot experiments 17.3 to 44.5% seedlings showed necrotic lesions on lower stem and roots and deformation of primary and secondary roots thus confirming the pathogenic nature of *F. oxysporum* on capsicum.

Table 3. Pathogenicity (Percentage infection) of *Fusarium solani* and *F. oxysporum* isolated from seed of capsicum. (400 seeds were used in each experiment)

	Fusarium solani			*Fusarium oxysporum*		
Categories	A	B	C	A	B	C
Healthy looking plants	36.0	30.0	12.0	31.0	38.0	35.0
Plants showing necrosis and deformation of roots	—	20.0	28.0	44.5	17.3	—
Dead plants	64.0	34.0	24.0	24.0	36.5	23.8
Rotted seeds	—	16.0	36.0	—	8.2	41.2

A: Roots of seedlings dipped in spore suspension and sown in sterilized sand, B: Seeds dipped in spore suspension and sown in sterilized sand, C: Seeds dipped in spore suspension and sown in agar slants.

The occurrence of saprophytes suggests that invasion of the seed by a fungus may occur independently of parasitism. The frequency of invasion by a particular fungus is possibly determined by the amount of inoculum in the seed environment. The ubiquity and preponderance of infection by *A. alternata*, a saprophytic species, lends support to this hypothesis. The comparative abundance of parasitic species of fungi in seed from Pakistan, Ghana, Turkey, Nigeria and India could be due to the moist weather conditions prevailing in these countries.

The three species of *Fusarium* viz., *F. moniliforme, F. Solani* and *F. oxysporum* caused seed rot and wilting of the seedlings. The effect of *F. moniliforme* was more pronounced under high humid conditions. It appears that the fungus prefers high moisture conditions because the capsicum seed sample collected from Gujranwala region in the province of the Punjab in Pakistan had 83.0% seed infection. The annual rainfall in Gujranwala district is generally higher than in other parts of the country. *F. moniliforme* is also known to kill young sorghum if cloudy humid conditions persist for an extended period (Zummo, 1980). It would suggest that seeds for cultivation and germ plasm bank should preferably be collected from areas where high humid conditions do not persist.

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