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Fungal Endophytes Survey of Some Legume Seeds

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Mycological survey of associated fungi with common bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*) seed samples, collected from markets throughout the Riyadh region of Saudi Arabia, was investigated. Frequencies of the isolated fungi were statistically compared. Seventeen and sixteen species belonging to eleven genera were isolated from common bean and cowpea respectively. *Aspergillus niger* and *A. flavus* were the highest distributed species (55.55%) over common bean sample locations. *A. niger* was also the highest distributed species (61.11%) over cowpea sample locations followed by *A. flavus* (58.33%) and *Fusarium* spp. (50%). *A. niger* was the most dominant in both crop seeds with 22.09% and 26.83% isolation frequencies of the total fungi of common bean and cowpea respectively. *A. flavus* was second and third most frequent fungus of the total fungi isolated from common bean (21.12%) and cowpea (18.48%) respectively. Significant positive and/or negative correlation were among some isolated fungi, implying the potential existence of synergism and/or antagonism among these fungi. Thus, more effort should be made to minimize the risk of endophytes accumulation in imported agricultural commodities.

Key words: *Phaseolus vulgaris*, Myco-contaminant, *Vigna unguiculata*, Pulses.

Among the major Pulses (food legumes) that imported to Kingdom of Saudi Arabia for food and/or baking purposes are common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata*). The former is the world's second most important bean after soybeans; and largely consumed throughout the world[1,2]. The latter is a rich source of proteins, minerals and vitamins, therefore it's mainly used, as a major cereal food diet in some countries[3,4] . Pulses generally, represent an important sources of dietary protein for human and animals. They are good sources of slow release carbohydrates, essential minerals, dietary fibers and vitamins and they have been reported to reduce blood cholesterol levels[5]. Moreover, Common bean consumption has been linked to reduced risks of cardiovascular disease, diabetes mellitus, obesity, cancer and digestive tract diseases[6].

Like various foodstuffs and agricultural commodities, fungal contamination of common bean and cowpea is a major problem and results in reduction in their qualities and nutritive values as well as human and livestock poisoning[7,8]. Many fungal genera; *Alternaria*, *Acremonium*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Mucor*, *Penicillum*, *Pythium*, *Rhizopus* and *Trichoderma* known to invade stored grains and legumes[9-12].

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Fungal contamination cause direct and/ or indirect reductions in the quality of foodstuffs and harm the human consumers. For example, *A. flavus* found to affect lipids and carbohydrates contents of plant seeds in addition to the aflatoxin production[13].

The aim of the current study was to isolate and identify the common myco-contaminants associated with common bean and cowpea seeds, commoditized in Kingdom of Saudi Arabia

MATERIALS AND METHODS

Sampling and Isolation

Thirty six samples of legume seeds; common bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*) collected from different locations in Riyadh city, Kingdom of Saudi Arabia were used for isolation and detection of the legume myco-contaminants.

Fungi were isolated and cultured according to the method described by Yassin *et al.*, (2011)[14]. Samples were surface-sterilized using 1% sodium hypochlorite solution and washed three times with sterile distilled water. Ten seeds in each case were placed randomly on the surface of Petri dishes containing Potato Dextrose Agar (PDA). Four replicate plates/sample were incubated at 25±2 ºC and examined daily for five days, after which the colonies developing from the seeds were counted. Isolated fungi were purified either by single spore or hyphal tip methods, and then transferred to slanted PDA. Isolates‘ identification was carried out in the Mycological Center, Assiut University, Egypt.

Statistical analysis

Correlation, and cluster analyses of the collected data of the fungal isolation frequencies were performed using SPSS 16 statistical package.

RESULTS

Mycological survey of fungi associated with common bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*) seed samples was investigated. Seventeen species and sixteen species belonging to eleven genera were recovered from common bean and cowpea seeds respectively. *A. niger* and *A. flavus* were the highest distributed species over common bean sample locations by 55.55% for both, while *Cladosporium sp.* was the lowest one (2.77%). Some fungal species such as *Botrytis* sp. and *Fusarium* spp. were equally (13.88%) distributed (Table 1).

Regarding cowpea samples, *A. niger* was also the highest distributed species (61.11%) over sample locations followed by *A. flavus* (58.33%) then *Fusarium* spp. (50%). Meanwhile, *Mucor sp.*,

Table 1. Distribution % of isolated fungi from common bean and cowpea over sample locations.

	Distribution % over locations Common bean fungi			Cowpea fungi	
F1	*Alternaria alternata*	30.55	F1	*Alternaria alternata*	19.44
F2	*Aspergillus flavus*	55.55	F2	*Aspergillus flavus*	58.33
F3	*A. f. far colunaris*	8.33	F3	*A. f. far colunaris*	11.11
F4	*A. niger*	55.55	F4	*Aspergillus niger*	61.11
F5	*A. ochracious*	5.55	F5	*A. parasitica*	5.55
F6	*Aspergillus sp.*	13.88	F6	*Aspergillus sp.*	19.44
F7	*Aspergillus ustus*	8.33	F7	*Aspergillus ustus*	5.55
F8	*Botrytis sp.*	13.88	F8	*Botryodiplodia sp.*	5.55
F 9	*Cheatomiun sp.*	8.33	F 9	*Cladosporium sp.*	5.55
F10	*Cladosporium sp.*	2.77	F10	*Curvularia sp.*	8.33
F11	*Fusarium spp.*	13.88	F11	*Fusarium spp.*	50.00
F12	*penicillium chrysogenum*	11.11	F12	*Mucor sp.*	2.77
F13	*penicillium spp.*	25.00	F13	*Nigrospora sp.*	2.77
F14	*Phoma sp.*	5.55	F14	*penicillium spp.*	8.33
F15	*Rhizopus stolonifer*	16.66	F15	*Rhizopus stolonifer*	13.88
F16	*Stemphyllium sp.*	5.55	F16	*Ulocladium sp.*	2.77
F17	*Ulocladium sp.*	5.55			

Table 2. Correlation coefficients among frequencies of fungi isolated from Common bean seeds. Isolates F1 to F17 as listed in Table 1

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17
F1	1	-0.346*	-0.159	-0.416*	0.167	-0.212	0.229	0.108	-0.075	-0.099	0.139	-0.078	0.136	0.390*	-0.169	-0.031	-0.049
F2		1	0.219	0.141	-0.147	0.215	-0.106	0.159	-0.166	0.191	0.281	-0.062	0.172	-0.087	-0.098	-0.247	-0.129
F3			1	0.137	-0.053	-0.098	-0.038	-0.108	-0.063	-0.046	-0.100	-0.087	-0.021	-0.065	-0.078	-0.062	-0.064
F4				1	0.064	-0.072	-0.100	-0.163	-0.121	-0.159	-0.032	-0.071	0.022	-0.078	0.080	-0.216	0.145
F5					1	-0.070	-0.063	-0.004	0.181	-0.033	-0.038	-0.062	-0.020	-0.046	-0.056	-0.045	-0.046
F6						1	0.031	0.161	-0.023	-0.061	0.045	-0.013	-0.073	-0.086	-0.104	-0.083	-0.086
F7							1	0.079	-0.076	-0.055	0.265	0.128	0.009	-0.077	-0.093	-0.074	-0.077
F8								1	-0.094	-0.068	0.142	-0.129	0.110	-0.096	-0.116	-0.092	-0.095
F9									1	-0.040	0.028	-0.075	-0.048	-0.056	-0.068	-0.054	-0.056
F10										1	-0.062-	-0.054	-0.084	-0.040	-0.049	-0.039	-0.040
F11											1	-0.118	0.115	-0.088	-0.106	-0.085	-0.088
F12												1	-0.159	-0.077	0.487**	-0.074	-0.076
F13													1	0.316	-0.143	-0.114	0.144
F14														1	-0.069	-0.055	-0.057
F15															1	-0.066	-0.069
F16																1	-0.055
F17																	1

Table 3. Correlation coefficients among frequencies of fungi isolated from Cowpea seeds. Isolates F1 to F16 as listed in Table 1

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16
F1	1	-0.179	0.015	-0.191	-0.109	0.240	-0.100	-0.113	-0.108	0.123	0.074	-0.080	-0.080	-0.067	-0.102	-0.080
F2		1	0.132	0.112	-0.081	-0.228	-0.181	-0.203	-0.015	-0.249	-0.286	0.278	0.077	0.561**	0.170	-0.145
F3			1	0.000	-0.103	-0.085	-0.095	-0.106	-0.102	-0.147	-0.140	0.083	0.403*	-0.164	0.219	-0.076
F4				1	-0.123	-0.082	-0.197	-0.221	-0.154	-0.206	-0.264	-0.090	0.088	0.030	-0.055	-0.158
F5					1	-0.109	0.082	-0.054	-0.052	-0.075	-0.144	-0.039	-0.039	-0.084	-0.076	-0.039
F6						1	-0.100	-0.113	-0.108	0.264	0.367*	-0.080	0.058	-0.128	0.048	-0.080
F7							1	-0.050	-0.048	-0.069	-0.151	-0.036	-0.036	-0.077	-0.070	0.960**
F8								1	-0.054	0.517**	0.055	-0.040	-0.040	-0.087	-0.078	-0.040
F9									1	-0.074	0.150	-0.038	-0.038	-0.083	-0.075	-0.038
F10										1	0.285	-0.055	-0.055	-0.069	-0.108	-0.055
F11											1	0.099	-0.153	-0.280	-0.022	-0.153
F12												1	-0.029	-0.062	-0.056	-0.029
F13													1	-0.062	-0.056	-0.029
F14														1	0.247	-0.062
F15															1	-0.056
F16																1
F17																

Nigrospora sp. and *Ulocladium* sp. were the least distributed fungi. On the other hand, some fungal species such as *A. alternata* and *Aspergillus* sp. were equally (19.44%) distributed (Table 1).

In respect of fungal isolation frequencies, *A. niger* were the most frequent fungus isolated from both crop seeds with 22.09% and 26.83% isolation frequencies of the total fungi of common bean and cowpea respectively. *A. flavus* was second and third most frequent fungus of the total fungi isolated from common bean (21.12%) and cowpea (18.48%) respectively (Fig. 1&3).

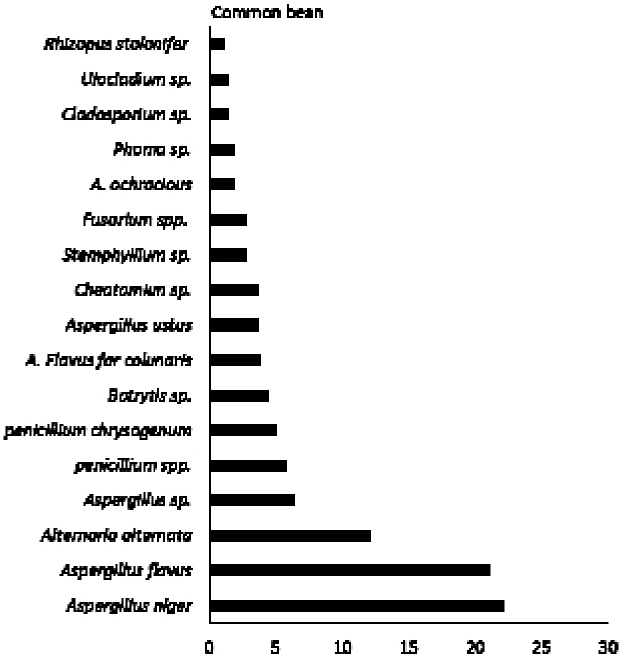

Fig. 1. Frequencies (%) of fungal species isolated from common bean seeds

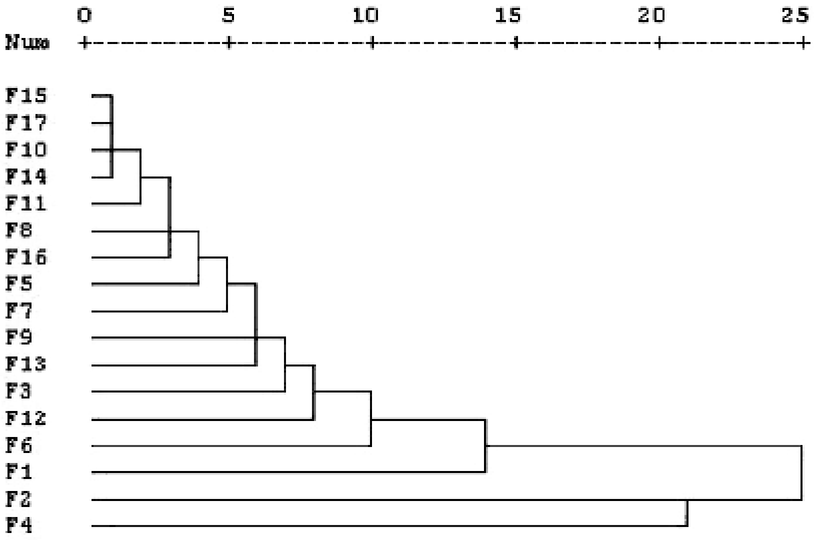

Fig. 2. Phenogram based on average linkage cluster analysis of frequencies of fungi recovered from common bean seeds. Isolates F1to F17 as listed in Table 1

The phenograms (Fig. 2 & 4) indicates that fungi isolated from both common bean and cowpea appear to form several distinct groups and subgroups based on their distribution patterns over samples. Within each group, fungi were associated strongly and positively in their distribution patterns over samples, whereas between groups, fungi were associated weakly or negatively. This result implies the potential existence of sample (environment) related groups of fungi.

Correlation analyses revealed that some of the isolated fungi were positively and/or negatively correlated with the others. Highly significant positive correlation was found among

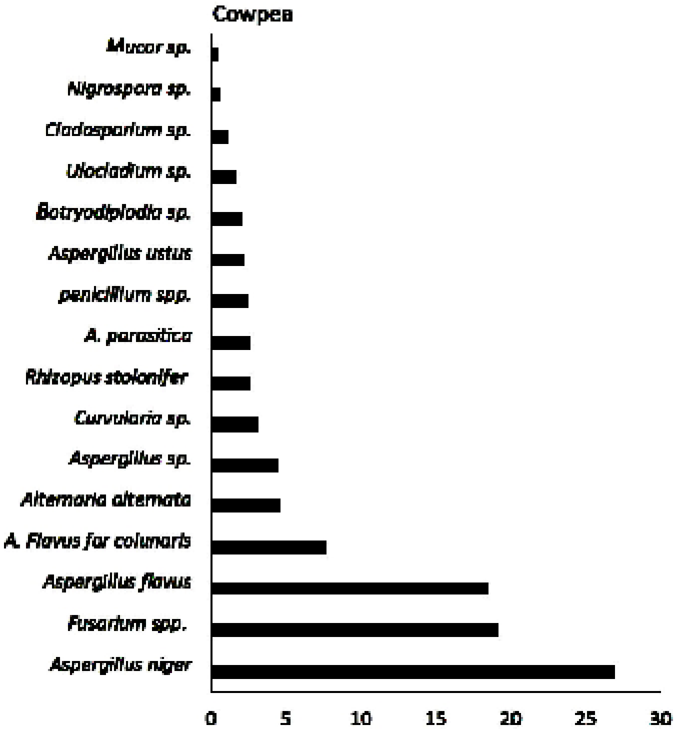

Fig. 3. Frequencies (%) of fungal species isolated from cowpea seeds

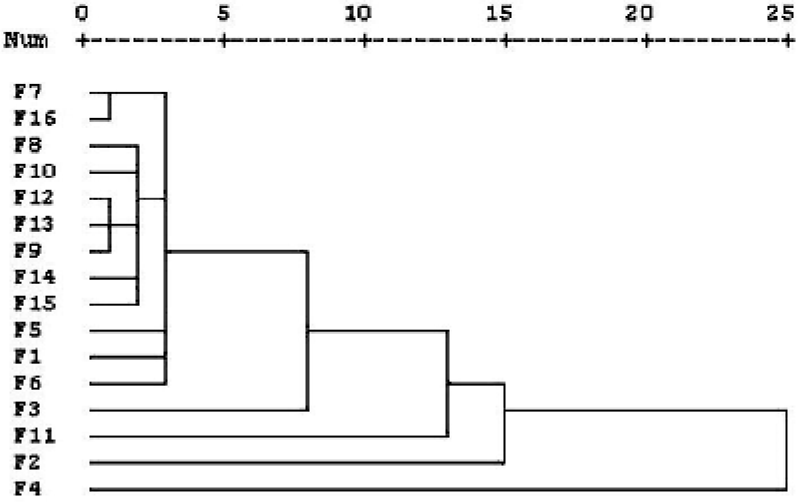

Fig. 4. Phenogram based on average linkage cluster analysis of frequencies of fungi recovered from cowpea seeds. Isolates F1to F16 as listed in Table 1

Penicillium chrysogenum and *Rhizopus stolonifer* in case of common bean associated fungi. On the other hand, significant negative correlation was among *A. alternata* and *A. niger* (Table 3). Regarding the cowpea associated fungi, highly significant positive correlation was found among *A. ustus* and *Ulocladium sp.* while, significant positive correlation was among *Aspergillus* sp. and *Fusarium* spp. (Table 4).

DISCUSSION

This study confirms the documented data that food cereals and other agricultural commoditize could be associated with serious fungal contaminants[15-18]. *Alternaria* spp., *Aspergillus* spp., *Botrytis* spp., *Chaetomium* spp., *Cladosporium* spp., *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp. and *Trichothecium* spp. were the most common fungi isolated from common bean in Croatia[19]. Meanwhile, common bean and two other Egyptian legume seeds had been contained several fungi belonging to four fungal genera; *A. flavus, A. niger, A. parasiticus, F. moniliforme, F. oxysporum, Fusarium* spp., *Penicillium* spp and *Sclerotinia sclerotiorum*[10,20]. All tested legume seeds including cowpea in an Indian investigation were contaminated with various fungi with the dominance of *A. flavus* in almost all seed samples[21]. Cowpea seed samples from South Africa and Benin had also been analyzed for seed mycoflora and found to be contaminated with seven different species belonging to *Fusarium* only[22].

Isolation of diverse fungal genera from legume seeds in the present study could be attributed to the ideal nutrient composition of such pulses[3,4,5] which make it a very good substrate for fungal growth[23,24]. Improper storage conditions might also play a critical role in pulses contamination and subsequently damages[25,26].

Dominance of some fungi such as *A. flavus*, *A. niger* and *Fusarium* spp. in this study confirms the earlier observations where *Aspergillus* spp. and *Fusarium* spp. were among the most predominant fungi in some of the stored pulses[27,28].

The cluster patterns of fungal distribution over samples, suggested that fungi were associated strongly and positively within each group, while they were associated weakly or negatively between groups. This results imply the potential existence of sample (environment) related fungi[14,16].

Highly significant positive correlation among some isolated fungi in this study, implying similar conditions for pulses colonization by such fungi. On the other hand, the significant positive correlation among some isolated fungi may indicate the possibility of synergism in colonizing the pulses. Meanwhile, a negative correlation may reflect an antagonism or competitive exclusion between such fungi[23,24,29].

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