

## Determination of Seedborne Fungi and Detection of Aflatoxins in Sudanese Fenugreek Seeds

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Following incubation of fenugreek (*Trigonella foenum-graecum* L.) seeds on potato dextrose agar and moist filter papers at  $28\pm 2^{\circ}\text{C}$ , 59 species and 11 varieties belonging to 21 genera of fungi were determined as seedborne in fenugreek crops. Among these isolates, 45 species and 9 varieties are new records for this crop; and two species are new to the mycoflora of Sudan: *Aspergillus stellifer* and *Emericella varicolor*. The genus *Aspergillus* (15 species and 8 varieties) is the most prevalent, followed by *Drechslera* (3 species), *Rhizopus* (3 species), *Alternaria* and *Fusarium* (6 species each), *Emericella* (4 species and 2 varieties), *Cladosporium* and *Penicillium* (4 species each), *Chaetomium* (3 species) and *Curvularia* (3 species and one variety). The remaining 11 genera displayed low level of infection. Of the common pathogens of fenugreek plants, *Fusarium oxysporum* (2.13%) was recovered from the seeds of this crop. Thin layer chromatographic analysis of chloroform extracts of 13 seed samples showed that two samples were naturally contaminated with aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> (7.5–35.2 µg/kg).

KEY WORDS: Aflatoxins; fenugreek; *Trigonella foenum-graecum*; seedborne fungi; Sudan.

### INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is a common spice crop of forage, with yields ranging between 150 and 275 kg/ha and seed yields between 500 and 3320 kg/ha. In the Sudan, fenugreek ('Helba') is grown under irrigation in Khartoum, Kordofan, the Nile and Northern states. Various species of fungi have been identified in fenugreek seeds produced under different climatic conditions (3,7,12,15,16). In Sudan, with its varied climatic conditions, various saprophytic and pathogenic fungi would be expected to be in association with different plants and plant products (5,6). However, no research has been conducted there on the seedborne mycoflora of fenugreek crops. The present study was therefore conducted to evaluate the mycoflora and occurrence of aflatoxins in fenugreek seeds collected from the local markets of Khartoum (Sudan).

### MATERIALS AND METHODS

Thirteen seed samples of fenugreek were obtained from the local markets of Khartoum during the harvest seasons of 1993 to 1997. The samples were taken and examined according to the International Seed Testing Association methods (11). Immediately after their collection, the moisture content was determined using the oven method. Replicates

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were ground in an electric mill and dried at 105°C to a constant weight. The moisture content was calculated as percentage of the initial weight.

TABLE 1. Incidence and number of fungal isolations<sup>z</sup> of fenugreek crops

Fungi isolated	New records <sup>y</sup>	Incidence %	
		Agar plate	Blotter test
<i>Alternaria alternata</i>		0.75 (3) <sup>z</sup>	ND (0)
<i>A. brassicicola</i>	*	7.88 (3)	3.75 (2)
<i>A. chlamydospora</i>	*	ND (0)	0.75 (2)
<i>A. citri</i>	*	5.88 (6)	ND (0)
<i>A. dianthi</i>	*	2.00 (3)	ND (0)
<i>A. tenuis</i>	*	0.50 (2)	2.63 (6)
<i>Aspergillus</i> sp.		1.25 (4)	0.50 (2)
<i>A. caespitosus</i>	*	1.88 (3)	ND (0)
<i>A. carbonarius</i>	*	6.00 (3)	2.00 (2)
<i>A. flavipes</i>		ND (0)	2.00 (1)
<i>A. flavus</i> var. <i>columnaris</i>		3.42 (12)	4.00 (7)
<i>A. flavus</i> var. <i>flavus</i>		4.13 (13)	1.63 (2)
<i>A. fumigatus</i>		0.56 (7)	0.50 (4)
<i>A. japonicus</i> var. <i>japonicus</i>	*	ND (0)	2.00 (1)
<i>A. nidulans</i> var. <i>echinulata</i>	*	2.00 (1)	ND (0)
<i>A. nidulans</i> var. <i>nidulans</i>	*	1.30 (9)	1.25 (3)
<i>A. niger</i>		7.59 (13)	5.83 (11)
<i>A. niger</i> var. <i>awamori</i>	*	ND (0)	6.00 (3)
<i>A. oryzae</i>	*	ND (0)	2.25 (4)
<i>A. parasiticus</i>		3.00 (4)	1.50 (2)
<i>A. stellifer</i>	*	1.25 (1)	ND (0)
<i>A. terreus</i> var. <i>aureus</i>	*	1.00 (1)	2.38 (4)
<i>A. terreus</i> var. <i>terreus</i>	*	2.35 (7)	ND (0)
<i>A. tetrazonus</i>	*	1.50 (3)	0.75 (1)
<i>A. unguis</i>	*	ND (0)	3.25 (2)
<i>Aureobasidium pullulans</i>	*	0.50 (1)	1.50 (2)
<i>Chaetomium elatum</i>	*	ND (0)	2.25 (2)
<i>C. globosum</i>		2.50 (6)	1.00 (1)
<i>C. spirale</i>	*	3.50 (3)	1.75 (2)
<i>Cladosporium cladosporioides</i>	*	0.75 (1)	2.50 (3)
<i>C. oxysporum</i>	*	0.92 (3)	1.25 (6)
<i>C. tenuissimum</i>	*	3.00 (3)	2.25 (2)
<i>C. uredinicola</i>	*	ND (0)	1.88 (4)
<i>Curvularia lunata</i>		ND (0)	3.75 (3)
<i>C. lunata</i> var. <i>aeria</i>	*	1.33 (3)	ND (0)
<i>C. pallescens</i>	*	ND (0)	0.50 (3)
<i>C. senegalensis</i>	*	1.00 (2)	1.13 (3)
<i>Drechslera australiensis</i>	*	3.33 (4)	4.25 (5)
<i>D. ellisii</i>	*	2.00 (2)	ND (0)
<i>D. spicifera</i>		8.00 (5)	14.75 (7)
<i>Emericella nidulans</i> var. <i>echinulata</i>	*	2.25 (1)	ND (0)
<i>E. nidulans</i> var. <i>nidulans</i>	*	1.00 (6)	1.25 (4)
<i>E. quadrilineata</i>	*	1.50 (2)	0.75 (2)
<i>E. unguis</i>	*	ND (0)	3.25 (2)
<i>E. varicolor</i>	*	1.25 (1)	ND (0)
<i>Fennellia flavipes</i>	*	ND (0)	2.00 (1)
<i>Fusarium</i> sp.		0.63 (4)	1.00 (1)
<i>F. chlamydosporum</i>	*	2.75 (3)	ND (0)
<i>F. oxysporum</i>		2.25 (2)	2.00 (2)

TABLE 1. (contd.)

<i>F. poae</i>		1.13 (4)	1.00 (1)
<i>F. pallidoroeseum</i>		0.50 (2)	0.25 (1)
<i>F. solani</i>		1.00 (1)	2.00 (2)
<i>F. verticillioides</i>		1.75 (5)	ND (0)
<i>Microascus trignonosporus</i>	*	ND (0)	1.75 (1)
<i>Mucor hiemalis</i>	*	4.50 (3)	ND (0)
<i>Penicillium</i> sp.		0.67 (3)	0.75 (1)
<i>P. chrysogenum</i>	*	0.75 (2)	0.25 (1)
<i>P. citrinum</i>	*	2.50 (4)	0.50 (1)
<i>P. funiculosum</i>	*	1.75 (2)	ND (0)
<i>P. purpurogenum</i>	*	1.88 (3)	2.00 (2)
<i>Phoma exigua</i>	*	0.25 (1)	0.88 (4)
<i>Pythium ultimum</i>	*	1.50 (1)	4.25 (2)
<i>Rhizopus arrhizus</i>		2.50 (3)	ND (0)
<i>R. oryzae</i>	*	2.00 (4)	0.75 (1)
<i>R. stolonifer</i>		5.55 (11)	8.50 (7)
<i>Sclerotium bataticola</i>	*	0.25 (1)	0.50 (3)
<i>Scopulariopsis trignonospora</i>	*	ND (0)	1.75 (1)
<i>Ulocladium</i> sp.		0.38 (3)	ND (0)
<i>Verticillium</i> sp.		1.08 (2)	ND (0)
Sterile mycelia (hyaline and dark)		1.25 (1)	2.13 (4)

<sup>z</sup>Number of fungal isolations, from 13 seed samples, shown in parentheses.

<sup>y</sup>\*, New records for fenugreek seeds.

ND, Not detected.

Isolation of fungi from the fenugreek seeds was carried out using routine agar plating and blotter methods. From each sample, 400 seeds were surface-disinfected in 0.1% mercuric chloride for 5 min and washed in several changes of sterile distilled water. The disinfected seeds were then incubated aseptically on potato dextrose agar at 28±2 °C for 1–2 weeks. Ten seeds were spaced according to their size on each petri dish. Similarly treated seed lots were inoculated on moist filter papers with cellulose wadding as blotters and incubated as above. The colonies of fungi which developed around the seeds on agar and filter papers were examined, identified microscopically and the average levels of contamination were recorded.

The bright greenish-yellow fluorescence method (2) and thin layer chromatography (TLC) (17) were used for determination of aflatoxins in 13 seed samples of fenugreek. Aflatoxin standards (Sigma: B<sub>1</sub>, A6636; B<sub>2</sub>, A9887; G<sub>1</sub>, A0138; G<sub>2</sub>, A0263) were used.

## RESULTS

The species determined in fenugreek seeds are presented in Table 1. Fifty-nine species and 11 varieties belonging to 21 genera of fungi were found to be seedborne for this crop. Among these isolates, 45 species and 9 varieties are new reports for fenugreek seeds, and two species are considered new to the mycoflora of Sudan: *Aspergillus stellifer* and *Emericella varicolor*.

The genus *Aspergillus* (41% of the total colony count of fungi) was the most common one, comprising 15 species and 8 varieties. This genus was followed by *Drechslera* (3 species, 12%), *Rhizopus* (3 species, 11%), *Alternaria* (6 species, 9%), and *Fusarium* (6 species), *Emericella* (4 species and 2 varieties), *Cladosporium* (4 species), *Penicillium* (4 species), *Chaetomium* (3 species) and *Curvularia* (3 species and one variety) (20%). The remaining 11 genera (one species each) together made up 7% of the contamination.

Some of the most common saprophytic and devastating pathogenic fungi of fenugreek plants were recovered from the seeds of this crop, and the seeds were highly contaminated with mold (13.25–56.0%, average 32.5%) (Table 2). Various species of toxigenic fungi of the genera *Aspergillus*, *Fusarium* and *Penicillium* were recovered from the seed samples. However, thin layer chromatographic analysis of chloroform extracts of the 13 seed samples showed that only two samples were naturally contaminated with aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> (7.5–35.2) µg/kg (Table 2).

TABLE 2. Contamination, moisture content (M.C.) and aflatoxins of 13 seed samples of fenugreek

Sample no.	Contamination (%)	M.C.%	Aflatoxins (µg/kg)
1	34.0	10.23	–
2	19.25	11.30	–
3	22.0	9.45	–
4	34.0	10.50	–
5	42.50	10.20	–
6	17.75	12.04	–
7	45.50	9.69	–
8	56.0	8.72	B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> and G <sub>2</sub> (7.5 µg/kg)
9	19.75	8.33	–
10	13.25	7.09	–
11	33.25	8.54	–
12	52.25	13.40	B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> and G <sub>2</sub> (35.2 µg/kg)
13	32.0	9.03	–

## DISCUSSION

Numerous species of fungi have been determined in fenugreek seeds cultivated in different climatic regions (3,7,12,15,16). In the present investigation, some of these fungi were recovered from the seeds of this crop. These include *Aspergillus flavipes*, *A. flavus* var. *columnaris*, *A. flavus* var. *flavus*, *A. fumigatus*, *A. niger*, *A. parasiticus*, *Chaetomium globosum*, *Curvularia lunata*, *Drechslera spicifera*, *Fusarium oxysporum*, *F. poae*, *F. pallidoroseum*, *F. solani*, *F. verticillioides*, *Rhizopus arrhizus*, *R. stolonifer*, *Ulocladium* sp. and *Verticillium* sp. Moreover, 45 species and 9 varieties of fungi were identified for the first time on fenugreek seeds, whereas *Aspergillus stellifer* and *Emericella variegata* are new to the mycoflora of Sudan. The occurrence of so many and so varied fungal species on these seeds suggested the high possibility of recovering additional new fungi, as indicated in similar studies (5,6).

In other pathogenicity studies of fenugreek plants (7,8), *Alternaria alternata* and *F. moniliforme* were the predominant fungi of fenugreek seeds, with *Botrytis cinerea*, *Curvularia inaequalis*, *C. lunata*, *Drechslera spicifera*, *Epicoccum purpurascens*, *F. oxysporum*, *F. pallidoroseum*, *F. solani*, *Phoma* sp., *Stemphylium botryosum*, *Ulocladium* sp. and *Verticillium albo-atrum* ranking second in abundance. In the present study the genus *Aspergillus*, which yielded 15 species and 8 varieties (41% of the total count of fungi), was the most prevalent genus, followed by *Drechslera*, *Rhizopus*, *Alternaria*, *Fusarium*, *Emericella*, *Cladosporium*, *Penicillium*, *Chaetomium* and *Curvularia*, while the remaining 11 genera exhibited very low levels of incidence. Similar results were obtained in mycological studies of some related legumes such as cowpea, lentil, lupine, pea and bean (1,4,5,6,9,10,14,18).

Fenugreek plants are vulnerable to infestation by different pathogenic seedborne fungi (12,15,16). In the present study, *F. oxysporum* was recovered (2.1%) from the seeds of this crop together with some destructive species of the common genera *Alternaria*, *Aspergillus*, *Chaetomium*, *Curvularia*, *Drechslera*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, and *Ulocladium* (5,6,14).

Pulses are prone to contamination with various mycotoxins, especially aflatoxins (4,5,6). The seeds of fenugreek are subject to attack by numerous toxin-producing species of the genera *Aspergillus* and *Fusarium* (3,8,13). In this research, the fenugreek seeds were naturally contaminated with various species of mold (13.2–56.0%, average 32.5%) and apparently infected by aflatoxigenic fungi such as *A. flavus* var. *columnaris*, *A. flavus* var. *flavus* and *A. parasiticus*. TLC analysis showed that two samples were naturally contaminated with aflatoxins B<sub>1</sub> and B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> at concentrations ranging between 7.5 and 35.2 µg/kg seeds. It is clear that the fairly dry conditions of Sudan under which the fenugreek seeds are generally stored favor mold development and aflatoxins production on the seeds.

In conclusion, it is evident that fenugreek seeds are naturally contaminated with many fungi, some of which are new reports for this crop and new to the mycoflora of Sudan. The study also demonstrated that these seeds harbor some of the saprophytic and potentially pathogenic fungi, which proved destructive under both field and storage conditions. The seeds of fenugreek seemed to be infected by a fair amount of common mold and aflatoxin-producing fungi. Therefore, special emphasis should be focused on harvesting techniques and storage methods to avoid the misuse of such contaminated seeds.

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