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# Composition of mycoflora and aflatoxins in pea seeds from the Sudan

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## ABSTRACT

Through incubation of 13 pea (*Pisum sativum* L. "Titan") samples from the local markets of Khartoum on potato dextrose agar (PDA) and moistened filter papers (Moist Chambers) at 28 ± 2°C, 22 genera, 56 species and 9 varieties were encountered as seed-borne fungi of pea crops. Of these fungi, 45 species and 9 varieties are new records to this crop, where two genera, three species and two varieties are new reports to the mycoflora of the Sudan. The genus *Aspergillus* (11 species and 5 varieties) was the most common followed by *Rhizopus* (2 species), *Alternaria* (7 species), *Fusarium* (7 species), *Emericella* (2 species and 3 varieties), *Drechslera* (2 species), *Cladosporium* (4 species) and *Penicillium* (5 species), where the remaining 14 genera (1–3 species) exhibited very low levels of infection. As possible pathogens of pea plants, *A. alternata* (2.07%), *A. flavus* var. *columnaris* (3.75%), *A. flavus* var. *flavus* (3.70%), C. *cladosporioides* (1.88%), *D. australiensis* (2.46%), *F. oxysporum* (1.58%), *F. solani* (1.88%) and *Pythium ultimum* (1.50%) were recovered from pea seeds. Thin layer chromatographic analysis of chloroform extracts of 13 seed samples revealed that three samples were naturally contaminated with aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ (18–30 μg/kg).

## INTRODUCTION

Pea (*P. sativum*) is a cool-season crop that has achieved popularity as a vegetable and pulse throughout the world since prehistoric times (Davies *et al.* 1985, Smart & Hymowitz 1985). In the Sudan, peas are grown in the Selaim Basin (Northern State), Shendi (Nile State) and Sennar (Middle State) (El Hassan 1984). The total area of the dry pea production harvested throughout the world is 8,060,000 ha. with a total production of about 14,529,000 metric tonnes giving an average yield of 1803 kg/ha. (FAO 1994). Pea production is apparently affected by infestation of various patho-

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genic seed-borne fungi (Moore 1946, Hussain & Yousuf 1967, Nelson *et al.* 1981, Maude *et al.* 1986, Agarwal & Sinclair 1987, Czyzewska 1987, Zhu & Zhang 1988, Bathgate *et al.* 1989, Gärber & Jahn 1990, Oyarzun *et al.* 1990, Allard *et al.* 1993, Kraft & Kaiser 1993, Santos *et al.* 1993, Schueler *et al.* 1993, Mills & Woods 1994). In the Sudan, the presence of different climatic conditions, a wide range of annual rainfall and various vegetation types suggested the high possibility of occurrence of numerous saprophytic and pathogenic fungi (Elshafie 1985, 1986), however, no study has been conducted in the Sudan on the seed-borne mycoflora of peas. Few studies were carried out on rusts, smuts, powdery mildews and leaf spotting diseases (Tarr 1955, 1963). Therefore, the present investigation was designed to evaluate the mycoflora and natural aflatoxins in pea seeds collected from the local markets of Khartoum (Sudan).

## MATERIALS AND METHODS

### Collection of seed samples

For testing pea seeds for their health conditions, 13 seed samples were purchased from the local markets of Khartoum. The samples represent the harvesting seasons of 1993–1996. The working samples were drawn and examined according to the International Rules for Seed Testing Association (ISTA 1966).

### Moisture content

Immediately after collection of the pea seed samples, moisture content for 13 seed samples was detected using the oven method (Zohri & Abdel Gawad 1992). Replicates of 100 seeds each were ground into flour and left to dry at 150°C to obtain a constant weight. The moisture content was calculated as a percentage of the initial weight.

### Isolation and estimation of seed-borne fungi

For the isolation of seed-borne fungi, routine agar plate and blotter methods were adopted (Christensen 1963, Hussain *et al.* 1989, Zohri & Abdel Gawad 1992, El-Kady & Youssef 1993, Moslem & Parvez 1993). In these methods, 800 seeds from each sample were disinfected with mercuric chloride (0.1%, 5 minutes), washed in several changes of sterile distilled water, and incubated aseptically on potato dextrose agar (PDA) and moistened filter papers (Moist Chambers). The inoculated plates were incubated at 28 ± 2°C for one to two weeks and were then examined using a stereoscopic binocular microscope to determine the natural growth of fungi on the seeds. The average levels of contamination of the seeds on moistened papers and PDA were recorded.

### Identification

Whenever possible, the identification of fungi on the seeds was carried out with the aid of the microscope. When this was not possible, the isolated fungi were incubated on various diagnostic growth media. The genus *Aspergillus* and other genera except *Fusarium* and *Penicillium* were incubated at 25 and 37°C on Czapek Dox agar (CDA) (Singh *et al.* 1991, Moubasher 1993), and at 25°C on Czapek yeast extract

agar (CYA) (Pitt 1973), Czapek yeast extract agar with 20% sucrose (CY20S) (Pitt & Hocking 1985) and malt extract agar (MEA) (Blakeslee 1915). The species of the genus *Fusarium* were incubated on wheat-seed agar (WSA) (Synder & Hansen 1947, Zacharaih *et al.* 1956, Tschanz *et al.* 1975), potato dextrose agar (PDA) and carnation-leaf agar (CLA) (Nelson *et al.* 1983). The seeded plates were incubated under laboratory conditions (25–33°C) for one week. The species of the genus *Penicillium* were incubated at 25°C on Czapek yeast autolysate agar (CYA) (Singh *et al.* 1991) and Czapek solution agar (CSA) (Raper & Thom 1968).

The identification of the isolated fungi was confirmed using many taxonomic papers and monographs (Bessy 1950, Barnett 1962, Raper & Fennell 1965, Raper & Thom, 1968, Ellis 1971, 1976, Nelson *et al.* 1983, Elshafie 1985, 1986, Klich & Pitt 1988, Singh *et al.* 1991, Moubasher 1993). Different publications of the Commonwealth Mycological Institute (CMI), the International Mycological Institute (IMI) and the Danish Government Institute of Seed Pathology for Developing Countries were also used.

### Aflatoxin analysis

The presence of aflatoxins in 13 seed samples of pea was primarily detected by bright greenish-yellow fluorescence (BGYF) (Fennell *et al.* 1973, Bothast & Hasseltine 1975). In this method, a portion of the seed sample was ground coarsely and observed under long wave ultraviolet (UV) light (365 nm) and short wave UV light (250 nm) fitted in a black cabinet. Thin layer chromatography (TLC) according to Thomas *et al.* (1975) and adapted by Singh *et al.* (1991) was used for the determination of aflatoxin contents. In this method, 1000 gm from each sample were powdered, 50 gm were added to 250 ml of ethanol:water (60:40 v/v) and shaken on a mechanical shaker for 30 minutes. The solution was left to sediment, then filtered and 125 ml of the filtrate were placed into a 250 ml separating funnel to which 30 ml of saturated sodium chloride and 50 ml hexane were added. The mixture was shaken vigorously for two minutes and left to separate. The lower methanol water layer was collected into another 250 ml separating funnel, 50 ml of chloroform were added and the mixture shaken with frequent venting to reduce pressure from vapourised chloroform. The chloroform layer was drained into a conical flask containing 5 gm of cupric carbonate, shaken, left to settle, filtered through Whatman filter paper No. 42 having a bed of anhydrous sodium sulphate, and the chloroform extract was collected into a beaker. The cupric carbonate residue was washed with 25 ml chloroform again and filtered through a sodium sulphate bed. The two chloroform extracts were combined, evaporated in a water bath until dry and the residue was dissolved in 1 ml of chloroform and transferred into a screw cap vial. The extract was kept for qualitative and quantitative estimation of aflatoxins. The aflatoxin standards used in the present study were kindly supplied by the National Research Centre, Food Research Centre and Ministry of Commerce (Sudan).

## RESULTS

The fungal species recovered from the seeds of pea are presented in Table 1. Fifty-six species and nine varieties which belong to 22 fungal genera were recorded as seed-borne fungi. From these isolates, 45 species and nine varieties are new reports

**Table 1.** Incidence, number of cases isolated (NCI, out of 26 replicates) and occurrence remarks (OR**) of fungi in pea seeds.

| Fungi isolated | | Incidence % | | | | |
|---|---|---|---|---|---|---|
| | [1]New records | Agar Plate | Blotter Test | Average | NCI | [2]OR** |
| *Alternaria alternata* | | 1.05 | 3.08 | 2.07 | 19 | H |
| *A. brassicicola* | é | 7.0 | 3.50 | 5.25 | 4 | L |
| *A. chlamydospora* | é* | 2.50 | – | 2.50 | 2 | R |
| *A. citri* | é* | 1.00 | – | 1.00 | 1 | R |
| *A. dianthi* | é | – | 5.50 | 5.50 | 3 | R |
| *A. tenuis* | é | 3.19 | – | 3.19 | 8 | M |
| *A. tenuissima* | é | – | 1.25 | 1.25 | 2 | R |
| *Aspergillus* sp. | | 0.88 | 0.25 | 0.57 | 6 | L |
| *A. caespitosus* | é* | 0.50 | 1.75 | 1.13 | 8 | M |
| *A. carbonarius* | é* | – | 6.00 | 6.00 | 3 | R |
| *A. flavus* var. *columnaris* | é* | 6.0 | 1.50 | 3.75 | 7 | M |
| *A. flavus* var. *flavus* | é | 1.65 | 5.75 | 3.70 | 17 | H |
| *A. fumigatus* | é | 1.00 | – | 1.00 | 5 | L |
| *A. hollandicus* | é* | – | 3.50 | 3.50 | 3 | R |
| *A. niger* | | 5.38 | 6.42 | 5.90 | 22 | H |
| *A. oryzae* | é* | 2.25 | – | 2.25 | 2 | R |
| *A. parasiticus* | é* | 2.00 | 2.50 | 2.25 | 4 | L |
| *A. sydowii* | é* | 0.75 | – | 0.75 | 1 | R |
| *A. terreus* var. *africanus* | é* | 0.50 | – | 0.50 | 3 | R |
| *A. terreus* var. *aureus* | é* | – | 1.25 | 1.25 | 1 | R |
| *A. terreus* var. *terreus* | é | 2.25 | 5.50 | 3.88 | 7 | M |
| *A. violaceo-brunneus* | é* | 1.75 | – | 1.75 | 4 | L |
| *Aureobasidium pullulans* | é | 2.5 | – | 2.50 | 3 | R |
| *Chaetomium* sp. | | 1.50 | 0.88 | 1.19 | 5 | L |
| *C. globosum* | é | 0.5 | 2.25 | 1.38 | 3 | R |
| *C. spirale* | é* | 0.25 | 1.25 | 0.75 | 5 | L |
| *Cladosporium aecidiicola* | é* | 0.88 | 1.50 | 1.19 | 5 | L |
| *C. cladosporioides* | | 1.75 | 2.0 | 1.88 | 6 | L |
| *C. oxysporum* | é* | 1.13 | 1.50 | 1.32 | 11 | M |
| *C. uredinicola* | é* | 1.50 | 1.50 | 1.50 | 5 | L |
| *Cunninghamella echinulata* | é | 1.00 | 2.50 | 1.75 | 4 | L |
| *Curvularia brachyspora* | é | – | 1.25 | 1.25 | 3 | R |
| *C. intermedia* | é | 1.00 | – | 1.00 | 1 | R |
| *C. lunata* | é | 0.75 | 2.00 | 1.38 | 5 | L |
| *C. lunata* var. *aeria* | é | 0.50 | 2.75 | 1.63 | 5 | L |
| *Drechslera australiensis* | é | 2.17 | 2.75 | 2.46 | 8 | M |
| *D. spicifera* | | 1.00 | 1.38 | 1.19 | 15 | H |
| *Emericella nidulans* var. *echinulata* | éá* | 8.25 | – | 8.25 | 2 | R |
| *E. nidulans* var. *lata* | é á* | 0.75 | – | 0.75 | 2 | R |
| *E. nidulans* var. *nidulans* | é | 2.08 | 1.50 | 1.79 | 9 | M |
| *E. violacea* | é* | 1.75 | – | 1.75 | 3 | R |
| *Eurotium amstelodami* | é | – | 3.50 | 3.50 | 2 | R |
| *Fusarium* sp. | | 1.38 | 0.25 | 0.81 | 7 | M |
| *F. chlamydosporum* | é | 1.13 | – | 1.13 | 5 | L |
| *F. equiseti* | | 0.25 | 0.50 | 0.38 | 3 | R |
| *F. graminearum* | * | 0.63 | 0.50 | 0.57 | 4 | L |

**Table 1.** *Continued.*

| Fungi isolated | [1]New records | Incidence % Agar Plate | Blotter Test | Average | NCI | [2]OR** |
|---|---|---|---|---|---|---|
| *F. oxysporum* | | 1.58 | – | 1.58 | 8 | M |
| *F. palidoroseum* | | 1.50 | – | 1.50 | 1 | R |
| *F. solani* | | 1.75 | 2.00 | 1.88 | 6 | L |
| *F. verticillioides* | é | 2.00 | – | 2.00 | 5 | L |
| *Mucor* sp . | | 2.50 | 1.50 | 2.00 | 2 | R |
| *M. hiemalis* | é | 1.50 | 3.75 | 2.63 | 6 | L |
| *Myrothecium verrucaria* | é | – | 2.50 | 2.50 | 1 | R |
| *Penicillium* sp. | | 0.75 | 0.25 | 0.50 | 4 | L |
| *P. chrysogenum* | é* | – | 1.00 | 1.00 | 4 | L |
| *P. citrinum* | é | 1.50 | – | 1.50 | 2 | 2R |
| *P. funiculosum* | é | 2.00 | 1.25 | 1.63 | 5 | L |
| *P. griseofulvum* | é* | 4.00 | – | 4.00 | 2 | R |
| *P. oxalicum* | é* | 1.13 | – | 1.13 | 1 | R |
| *Phoma* sp. | | 0.38 | 0.50 | 0.44 | 2 | R |
| *P. herbarum* | é | – | 1.50 | 1.50 | 2 | R |
| *Pythium* sp. | | 0.63 | 0.25 | 0.44 | 7 | M |
| *P. ultimum* | á* | – | 1.50 | 1.50 | 3 | R |
| *Rhizopus* sp. | | 1.38 | – | 1.38 | 2 | R |
| *R. arrhizus* | é | 2.00 | – | 2.00 | 2 | R |
| *R. stolonifer* | | 4.34 | 8.25 | 6.30 | 25 | H |
| *Scytalidium lignicola* | éá* | 2.25 | – | 2.25 | 3 | R |
| *Thermomyces lanuginosus* | é* | – | 0.50 | 0.50 | 1 | R |
| *Ulocladium* sp. | | 0.88 | – | 0.88 | 2 | R |
| *U. botrytis* | é | – | 3.0 | 3.00 | 3 | R |
| *Verticillium* sp. | * | 1.50 | – | 1.50 | 2 | R |
| *V. tenerum* | é* | – | 1.25 | 1.25 | 3 | R |
| *Sterile mycelia* | | 0.50 | 0.75 | 0.63 | 6 | L |

[2]OR**: Occurrence remarks: out of 26.
H: high, more than 13 replicates.
M: moderate, between 7-13 replicates.
L: low, between 4-6 replicates.
R: rare, less than 4 replicates.

[1]*: Reported in the Sudan by El-Nagerabi (1997).
*é*: New records for pea seeds.
á: New reports for the Sudan.

of the mycoflora on pea seeds. These include the genus *Aspergillus* (10 species and 5 varieties), *Alternaria* (6 species), *Penicillium* (5 species), *Curvularia* (3 species and one variety), *Cladosporium* (3 species), *Emericella* (2 species and 3 varieties), *Chaetomium* and *Fusarium* (2 species each), *Aureobasidium*, *Cunninghamella*, *Drechslera*, *Eurotium*, *Mucor*, *Myrothecium*, *Phoma*, *Rhizopus*, *Scytalidium*, *Thermomyces*, *Ulocladium* and *Verticillium* (one species each). *Emericella nidulans* var. *echinulata*, *E. nidulans* var. *lata*, *Pythium ultimum* and *Scytalidium lignicola* are new records to the mycoflora of the Sudan (Table 1).

The genus *Aspergillus* (34% of the total colony count of fungi) was the most common, comprising 11 species and 5 varieties. This genus was followed by *Rhizopus* (2 species, 17%), *Alternaria* (7 species, 12%), *Fusarium* (7 species), *Emericella* (2 species

**Table 2.** Contamination (C.%), moisture content (M. C.%) and aflatoxins of 13 seed samples of pea.

| Sample No. | C. % | M. C.% | Aflatoxins |
|---|---|---|---|
| 1 | 16.25 | 10.96 | –ve |
| 2 | 34.75 | 10.30 | –ve |
| 3 | 33.75 | 9.43 | –ve |
| 4 | 9.50 | 9.55 | -ve |
| 5 | 47.75 | 9.69 | +ve $B_1$, $B_2$, $G_1$ and $G_2$ (24 μg/kg). |
| 6 | 45.25 | 10.72 | –ve |
| 7 | 25.00 | 9.20 | +ve $B_1$, $B_2$, $G_1$ and $G_2$ (18 μg/kg). |
| 8 | 29.25 | 9.53 | –ve |
| 9 | 22.25 | 11.70 | –ve |
| 10 | 13.00 | 9.55 | –ve |
| 11 | 25.25 | 8.02 | –ve |
| 12 | 47.50 | 10.09 | +ve $B_1$, $B_2$, $G_1$ and $G_2$ (30 μg/kg). |
| 13 | 18.00 | 8.52 | –ve |

and 3 varieties), *Drechslera* (2 species), *Cladosporium* (4 species) and *Penicillium* (5 species) (23%), where the remaining 14 genera (1–3 species) exhibited very low levels of infection (14%).

Some of the possible pathogens of the pea plants were recovered from the seeds of this crop and the seeds were highly contaminated with mould fungi (9.5–47.75%, average 28.31%) (Table 2).

Various species of the mycotoxigenic fungi of the genera *Aspergillus*, *Fusarium* and *Penicillium* were recovered from the seed samples of this crop. Thin layer chromatographic analysis of the chloroform extracts of the 13 seed samples (Table 2) showed that three samples were naturally contaminated with aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ (18–30 μg/kg).

## DISCUSSION

### Mycoflora of pea seeds

Examination of pea seeds incubated on potato dextrose agar (PDA) and moistened filter papers (Moist Chambers) at 28 ± 2°C revealed that the pea seeds were infected with 56 species and nine varieties which belong to 22 fungal genera. Various species of fungi have been isolated from pea seeds produced in different climatic regions (Moore 1946, Hussain & Yousuf 1967, Nelson *et al.* 1981, Maude *et al.* 1986, Agarwal & Sinclair 1987, Czyzewska 1987, Zhu & Zhang 1988, Bathgate *et al.* 1989, Gärber & Jahn 1990, Oyarzun *et al.* 1990, Allard *et al.* 1993, Kraft & Kaiser 1993, Santos *et al.* 1993, Schueler *et al.* 1993, Mills & Woods 1994). In the present study, some of these fungi were recovered from the seed samples of pea crop. These include *Aspergillus niger* (5.90%), *Fusarium equiseti* (0.38%), *F. oxysporum* (1.58%), *F. palidoroseum* (1.50%), *F. solani* (1.88%), *Pythium ultimum* (1.50%) and *Rhizopus stolonifer* (6.30%). Forty-five species and nine varieties are new reports of the mycoflora of pea seeds. Among these *Alternaria brassicicola* (5.25%), *A. chalmydospora* (2.50%), *A. citri* (1.0%), *A. dianthi* (5.50%), *A. tenuis* (3.19%), *A.*

*tenuissima* (1.25%), *Aspergillus caespitosus* (1.13%), *A. carbonariius* (6.0%), *A. flavus* var. *columnaris* (3.75%), *A. flavus* var. *falvus* (3.70%), *A. fumigatus* (1.0%), *A. hollandicus* (3.50%), *A. oryzae* (2.25%), *A. parasiticus* (2.25%), *A. sydowii* (0.75%), *A. terreus* var. *africanus* (0.50%), *A. terreus* var. *aureus* (1.25%), *A. terreus var. terreus* (3.88%), *A. violaceo-brunneus* (1.75%), *Aureobasidium pullulans* (2.50%), *Chaetomium globosum* (1.38%), *C. spirale* (0.75%), *Cladosporium aecidiicola* (1.19%), *C. oxysporum* (1.32%), *C. uredinicola* (1.50%), *Cunninghamella echinulata* (1.75%), *Curvularia brachyspora* (1.25%), *C. intermedia* (1.0%), *C. lunata* (1.38%), *C. lunata* var. *aeria* (1.63%), *Drechslera australiensis* (2.46%), *Emericella nidulans* var. *echinulata* (8.25%), *E. nidulans* var. *lata* (0.75%), *E. nidulans* var. *nidulans* (1.79%), *E. violacea* (1.75%), *Eurotium amstelodami* (3.50%), *Fusarium chlamydosporum* (1.13%), *F. verticillioides* (2.0%), *Mucor hiemalis* (2.63%), *Myrothecium verrucaria* (2.50%), *Penicillium chrysogenum* (1.0%), *P. citrinum* (1.50%), *P. funiculosum* (1.63%), *P. griseofulvum* (4.0%), *P. oxalicum* (1.13%), *Phoma herbarum* (1.50%), *Rhizopus arrhizus* (2.0%), *Scytalidium lignicola* (2.25%), *Thermomyces lanuginosus* (0.50%), *Ulocladium botrytis* (3.0%) and *Verticillium tenerum* (1.25%). *Emericella nidulans* var. *echinulata* (8.25%), *E. nidulans* var. *lata* (0.75%), *Pythium ultimum* (1.50%) and *Scytalidium lignicola* (2.25%) are new records to the mycoflora of the Sudan. The occurrence of these numerous and variable fungi on these seeds indicated the high possibility for further recovery of many other saprophytic and pathogenic fungi as suggested by Elshafie (1985, 1986).

Rattan (1974) and Neergaard (1977) stated that *Fusarium oxysporum* f. sp. *pisi* is one of the major seed-borne fungi of pea seeds. Field and King (1962) concluded that non-inoculated high quality peas stored at 10–30°C and 75–92% relative humidity for two to eight months remained free from *Aspergillus*. Mycological studies of peas and other related leguminous crops (lupine, bean, cowpea, soybean, lentil) showed that the genus *Aspergillus* was the most prevalent genus, where the other genera display variable levels of infection (Moubasher *et al.* 1979b, Hitokoto *et al.* 1981, Abdel-Hafez & Shoreit 1986a, Hussain *et al.* 1989, El-Kady & Youssef, 1993, Moslem & Parvez 1993). In the Sudan, Abdel Wahab (1986) found that the infection levels of seed-borne fungi of some leguminous crops (*Cajanus cajan, Cicer arietinum, Dolichos lablab, Medicago sativa, Phaseolus vulgaris, Vigna unguiculata*) are very low. However, the species of the genera *Aspergillus* and *Rhizopus* exhibited infection levels between 0.1–42% and 0.5–60%, respectively. In the present investigations, the genus *Aspergillus* was the most common, followed by *Rhizopus, Alternaria, Fusarium, Emericella, Drechslera, Cladosporium* and *Penicillium*, where the remaining 14 genera exhibited very low levels of infection.

Pea production is significantly affected by infestation by various pathogenic seed-borne fungi (Hassan 1970, Czyzewska 1987). It was stated that *F. oxysporum* f. sp. *pisi* is one of the major seed-borne diseases of pea plants (Rattan 1974, Neergaard 1977). *A. alternata, C. cladosporioides, Epicoccum purpurascens, Stemphylium botryosum* and *Sordaria fimicola* were the dominant disease-causing pathogens on leaves and pods of pea plants (Furgal 1990/1991). The root rot complex caused by *Rhizoctonia solani, Fusarium solani, F. oxysporum* f. sp. *pisi, Aphanoascus euteiches* and *Pythium ultimum* has been reported from all commercial pea growing areas of the world (Ali *et al.* 1993/94). On the other hand, the diseases that attacked peas at seed and harvest stage were seed rots (*R. solani, Pythium* spp.), root rots (*F. solani* f. sp. *pisi, A. euteiches*), wilt (*F. oxysporum* f. sp. *pisi*), foliar

disease (*Phoma medicaginis* var. *pinodella*), powdery mildew (*Erysiphe pisi*) and downy mildew (*Peronospora viciae*) (Kraft & Kaiser 1993). *Phoma medicaginis* var. *pinodella* caused yield reduction of 25.0% (Wallen 1965). Cross inoculation of *Drechslera australiensis* showed that this fungus was pathogenic to different crops including pea (Kapoor & Tandon 1969). *Macrophomina phaseolina* was reported by Ali & Dennis (1992) as a serious pathogen of field peas causing leaf, stem, and petiole lesions. *E. pisi* (powdery mildew) is a seed-borne pathogen which caused disease to plants grown from untreated seeds of pea (Crawford 1927, Alcock 1931, Uppal *et al.* 1935). In addition, the powdery mildew caused by *E. polygoni* resulted in a high reduction in pea growth and yield parameters (Rathi & Tripathi 1994). In the Sudan, *Leveillula taurica* and *E. polygoni* caused a considerable loss in the production of pea crops (Boughey 1946a,b). Huang *et al.* (1992) proved that dry pea is highly susceptible to infection with *Pythium ultimum*. Of the genus *Aspergillus*, *A. flavus* was reported to be the most pathogenic species to pea plants (Fields & King 1962). In the present study, some of these possible pathogens have been isolated from the seeds of pea in very low levels of infection. These include *Alternaria alternata* (2.07%), *A. flavus* var. *columnaris* (3.75%), *A. flavus* var. *flavus* (3.70%), *Cladosporium cladosporioides* (1.88%), *Drechslera australiensis* (2.46%), *Fusarium oxysporum* (1.58%), *F. solani* (1.88%) and *P. ultimum* (1.50%). Of these fungi, *A. flavus* var. *columnaris*, *A. flavus* var. *flavus*, *Drechslera australiensis* are new reports of the mycoflora of the pea seeds, whereas *P. ultimum* is a new record to the mycoflora of the Sudan.

Although common genera like *Alternaria*, *Aspergillus*, *Chaetomium*, *Curvularia*, *Drechslera*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, and *Ulocladium* are known to be saprophytic in nature, some species of these genera can cause serious plant diseases (Moslem & Parvez 1993). In the present study, many species of these genera were recovered from pea seeds.

### Natural occurrence of aflatoxins in pea seeds

Pulses are subjected to considerable contamination with many mycotoxins, especially aflatoxins (Habish 1972, FAO 1979, Hitokoto *et al.* 1981, El-Maraghy 1989). In the present investigation, the seed samples of pea crops were found to be infected with various species of mould (9.50–47.75%, average 28.30%) and considerably contaminated with *A. flavus* var. *columnaris* (3.75%), *A. flavus* var. *flavus* (3.70%) and *A. parasiticus* (0.75%). Thin layer chromatographic analysis of the chloroform extract of the 13 seed samples showed that the seeds of pea were naturally contaminated with aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ (18–30 $\mu$g/kg) confirming the above findings. However, it is evident that the fairly dry conditions under which the pea seeds are generally stored in the Sudan does not favour much mould growth and aflatoxin production.

## CONCLUSION

From the above study it is pertinent to say that the seeds of pea crops are infected with numerous fungi, some of which are new for this crop. These include the genus *Aspergillus* (10 species and 5 varieties), *Alternaria* (6 species), *Penicillium* (5 species), *Curvularia* (3 species and one variety), *Cladosporium* (3 species), *Emericella* (2

species and 3 varieties), *Chaetomium* and *Fusarium* (2 species each), *Aureobasidium*, *Cunninghamella*, *Drechslera*, *Eurotium*, *Mucor*, *Myrothecium*, *Phoma*, *Rhizopus*, *Scytalidium*, *Thermomyces*, *Ulocladium* and *Verticillium* (one species each). Of these isolates, *Emericella nidulans* var. *echinulata*, *E. nidulans* var. *lata*, *Pythium ultimum* and *Scytalidium lignicola* are new records to the mycoflora of the Sudan. The investigations also revealed that the pea seeds harbour some possible pathogens such as *Alternaria alternata*, *A. flavus* var. *columnaris*, *A. flavus* var. *flavus*, *Cladosporium cladosporioides*, *Drechslera australiensis*, *Fusarium oxysporum*, *F. solani* and *P. ultimum* which may prove destructive under favourable conditions. Therefore, study of the etiology and pathogenicity of the seed-borne fungi is a priority in order to produce healthy crops. The seeds of peas are infected with considerable amounts of common moulds, some of which are known to be mycotoxigenic. This indicates the need for more attention to the harvesting and storage of dry peas in order to avoid the consumption and use of such contaminated seeds. The incidence of these fungi is however low to warrant such recommendation at present.

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# الفطريات والأفلاتكسنات في بذور البسلة من السودان

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## خلاصة

خلال زراعة 13 عينة من البسلة (*Pisum sativum* L. "Titan") جمعت من أسواق الخرطوم المحلية على وسط أجار البطاطس والدكستروز potato dextrose agar (PDA) و أوراق ترشيح مبللة (Moist Chambers) تحت 2±28 درجة مئوية ، وجد أن 22 جنس ، 56 نوع و 9 أضرب عبارة عن فطريات محمولة ببذور محصول البسلة . من هذه الفطريات 45 نوع و 9 أضرب عبارة عن سجلات جديدة لهذا المحصول ، بينما 2 جنس ، 3 نوع و ضربين عبارة عن سجلات جديدة بالنسبة للسودان . جنس أسبرجلس *Aspergillus* (11 نوع و 5 أضرب ) هو الأكثر شيوعاً يليه الرايزوبس *Rhizopus* (2 نوع) ، أولترناريا *Alternaria* (7 نوع) ، فيوزاريوم *Fusarium* (7 نوع) ، أميرسيلا *Emericella* (2 نوع و 3 أضرب) درجسليرا *Drechslera* (2 نوع) ، كلادوسبوريوم *Cladosporium* (4 نوع) وبنسيليوم *Penicillium* (5 نوع) ، بينما الأربعة عشر جنساً الباقية (1-3 نوع) أظهرت مستوى إصابة منخفض جداً. من الفطريات التي تسبب إصابة خطيرة لنباتات البسلة ، تم عزل فطر أولترنيريا أولترناتا *A.alternata* (2.07%) ، أسبرجلس فليفيس ضرب كولماريس *A. flavus* var.*columnaris* (3.75%) ، أسبرجلس فليفيس ضرب فليفيس *A. flavus* var. *flavus* (3.70%) كلادوسبوريوم كلادوسبوريويدس *C. cladosporioides* (1.88%) درجسليرا أستراينسيس *D. australiensis* (2.46%) ، فيوزاريوم أوكسيسبورم *F. oxysporum* (1.58%) ، فيوزاريوم سولانى *F. solani* (1.88%) وبثيوم التيمم *Pythium ultimum* (1.50%) من بذور البسلة . أوضح التحليل الكروماتوجرافي لخلاصة الكلوروفورم لبذورالبسلة أن البسلة ملوثة طبيعياً بأفلاتكسن ب$_1$ ، ب$_2$ ، ج$_1$ و ج$_2$ (18-30 ميكروجرام/كيلوجرام من البذور).