Phytopathol. Mediterr. (2000) 39, 257-262

Composition of mycoflora and aflatoxins in lupine seeds from the Sudan

Seifeldin Ahmed Faragalla El-Nagerabi and Abdulqader Elzein Elshafie[1]

Department of Microbiology, Faculty of Science, Taiz University, The Republic of Yemen
[1]Department of Biology, College of Science, Sultan Qaboos University, Muscat, Sultanate of Oman

Summary. Thirteen seed samples of lupines (*Lupinus termis* Forrsk.) were evaluated for their phytosanitary status by incubation on potato-dextrose agar (PDA) and moist sterile filter papers (blotter method) at 28±2°C. The seeds were also assayed for the presence of aflatoxins and toxigenic fungi. Forty-seven species and 10 varieties in 14 genera of fungi were recovered. Among these, 45 species and 10 varieties were new to lupine seeds, and 5 species and 2 varieties were new to the mycoflora of the Sudan. The genus *Aspergillus* (10 species, 8 varieties) was the most common, followed by *Rhizopus* (1 species), *Fusarium* (6 species) and *Alternaria* (5 species), while the remaining genera (*Chaetomium, Cladosporium, Curvularia, Drechslera, Penicillium, Phoma, Emericella, Mucor, Sclerotium, Ulocladium*) displayed lower levels of contamination. Of possible pathogens on lupine plants, *Alternaria* (5 species) (stem lesion) and *F. oxysporum* (root rot) were recovered from the seeds. Thin-layer chromatographic analysis of chloroform extracts of the 13 seed samples revealed that two samples contained very low concentrations of aflatoxins B_1 and B_2 (4.5-6.5 µg/kg).

Key words: aflatoxins, lupine, *Lupinus termis*, mycoflora, pathogens.

Introduction

The lupine (*Lupinus termis* Forrsk.) is an annual herb belonging to the tribe *Genisteae* (*Fabaceae*). It is an important crop in unfertile soils, where it is superior to cereals (Pate *et al.*, 1985). In the Sudan, it is grown mainly on the sandy soils in Darfur and Kordofan States (Western Sudan). This crop is subject to infection from various seedborne fungi (Gärber and Jahn, 1990). Some of these moulds secrete different types of mycotoxins in the seeds. Such toxins and especially aflatoxins cause various mycotoxicosis to man and his domestic livestock (Zohri and Abdel Gawad, 1992). In the Sudan, with its diversity in climatic conditions, rainfall and vegetation, large numbers of pathogenic and saprophytic fungi are expected to occur (Elshafie, 1986). However, little is known about the rusts, smuts, powdery mildews and leaf spot diseases in this country (Tarr, 1963).

Lupine production in the Sudan as that of other crops, is characterized by poor sanitary practices, low seed production and a scarcity of research. The present study was carried out to improve this situation.

Corresponding author: El-Nagerabi S.A.F.
Fax: + 967 4 203101
E-mail: nagerabi@hotmail.com

Materials and methods

Collection of seed samples

Thirteen seed samples of lupine (1 kg each) were obtained from local markets in Khartoum. The samples were collected randomly during the study

period and represented the harvesting seasons from 1993 to 1996. The samples were stored in polyethylene bags in refrigerator at 5°C and were drawn and examined in accordance with the international rules of the Seed Testing Association (ISTA, 1966).

Moisture content determination

From each sample lot, four replicates of 100 seeds were ground in an electric grinder and the flour was dried at 105°C to constant weight. The moisture content was calculated as a percentage of the initial weight.

Estimation of fungi and seed germination assays

Eight-hundred seeds from each sample were surface disinfected with mercuric chloride (0.1% in water) for 5 min, washed in several changes of sterile distilled water and incubated on potato-dextrose agar (PDA) or on sterile filter paper (blotter method) at 28±2°C. In each plate, eight seeds were spaced according to their size. Ten days later, the colonies of fungi, which had developed around the seeds, were examined and identified. The percentage of infected seeds and all fungal species isolated were recorded.

Aflatoxins assays

The bright greenish-yellow fluorescence method (BGYF) (Bothast and Hasseltine, 1975) and thin-layer chromatography (TLC) of chloroform extracts from seeds (Singh *et al.*, 1991) were used for detection of aflatoxins in the 13 seed samples of lupine. For aflatoxins extraction, 500 g from each sample were powdered, 50 g 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, 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 solution was vigorously shaken for 2 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 g 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 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.

Results

Estimation of fungi and seed germination assays

The fungal species isolated from lupine seeds are listed in Table 1. Forty-seven fungal species and 10 varieties in 14 genera were recorded as seed-borne fungi. Forty-five species and 10 varieties were new records for lupine seeds, and 5 species and 2 varieties are reported for the first time in the Sudan. The genus *Aspergillus* was predominant, yielding 10 species and 8 varieties (42% of the total fungal colony count). This genus was followed by *Rhizopus* (one species, 35%), *Fusarium* (6 species) and *Alternaria* (5 species) (both 7%), while the remaining 10 genera displayed very low levels of infection (16%). Many saprophytes and some of the potential pathogens of lupine plants were isolated from the seeds, which were highly contaminated (12.75-59.75%) and displayed varying levels of germination (62.0-99.5%, average 82.15%) (Table 2).

Toxigenic fungi and aflatoxin assays

Numerous species of toxin-producing fungi of the genera *Aspergillus*, *Fusarium* and *Penicillium* were recovered from the seeds of this crop. TLC of chloroform extracts of the 13 seed samples (Table 2) showed that 2 samples were naturally contaminated with aflatoxins B_1 and B_2, at concentrations ranging from 4.5 to 6.5 µg/kg of seeds.

Discussion

Various authors have isolated many fungi from the seeds of lupine in diverse climatic regions (Hitokoto *et al.*, 1981; Abdel-Hafez and Shoreit, 1986; El-Maraghy, 1989). In the present study, 47 species and 10 varieties in 14 genera were found to have contaminated the lupine crop. In previous mycological studies on lupine and other legumes:

Table 1. Infection counts of fungi in lupine seeds tested by the blotter method and on potato-dextrose agar.

Fungus	Type of record[a]	Recovery of fungi from lupine seeds (%)		Recovery (average) (%)	NIC[b]	Occurrence[c] remark
		From dishes with PDA	From dishes with wet filter paper			
Alternaria alternata	A	1.00	2.50	1.75	8	M
A. brassicicola	A	0.00	1.25	0.63	2	R
A. chlamydospora	AB	0.00	1.25	0.63	3	R
A. tenuis	A	1.33	0.00	0.67	5	L
A. tenuissima	A	0.00	3.25	1.63	3	R
Aspergillus sp.	B	0.38	1.00	0.69	6	L
A. carbonarious	AB	0.00	2.25	1.13	3	R
A. flavus var. *columnaris*	AB	9.63	4.90	7.27	14	H
A. flavus var. *flavus*	A	5.88	5.92	5.90	20	H
A. fumigans	A	0.25	1.13	0.69	5	L
A. japonicus var. *aculeatus*	AC	0.50	2.00	2.25	4	L
A. japonicus var. *japonicus*	AC	3.75	0.50	2.13	4	L
A. nidulellus var. *nidulans*	A	1.75	2.75	2.25	6	L
A. niger	A	7.36	5.40	6.38	24	H
A. niger var. *awamori*	AB	0.00	0.50	0.25	2	R
A. oryzae	AB	1.75	1.13	1.44	10	M
A. parasiticus	AB	0.75	0.00	0.38	3	R
A. terreus var. *aureus*	AB	2.00	1.25	1.75	6	L
A. terreus var. *terreus*	A	1.25	0.00	0.63	7	M
A. violaceo-brunneus	AB	0.75	0.00	0.38	2	R
Chaetomium sp.	B	1.25	0.38	0.82	3	R
C. elatum	AB	0.75	0.00	0.38	2	R
C. globosum	A	0.88	0.00	0.44	6	L
C. spirale	AB	1.25	2.63	1.94	7	M
Cladosporium herbarum	B	0.00	1.13	0.57	2	R
C. oxysporum	AB	1.13	1.75	1.44	11	M
C. sphaerospermum	A	0.38	1.00	0.69	7	M
Curvularia eragrostidis	A	1.25	1.00	0.63	4	L
C. intermedia	A	1.00	0.63	0.82	4	L
C. lunata	A	0.94	0.00	0.47	9	M
C. lunata var. *aeria*	A	1.00	0.75	0.88	4	L
C. pallescens	A	0.50	0.00	0.25	1	R
C. senegalensis	A	0.00	2.00	1.00	1	R
Drechslera australiensis	A	0.00	1.25	0.63	2	R
D. ellissi	A	1.00	1.38	1.19	8	M
D. hawaiensis	A	0.00	1.25	0.63	3	R
D. spicifera	A	0.88	2.50	1.69	6	L
Emericella nidulans var. *nidulans*	A	1.75	2.75	2.25	6	L
E. violacea	AB	0.75	0.00	0.38	2	R
Fusarium sp.	B	1.88	0.25	1.07	5	L
F. chlamydosporum	AC	0.00	7.00	3.50	2	R
F. compactum	AC	1.50	1.00	1.25	3	R
F. oxysporum	B	0.00	0.75	0.38	3	R
F. graminearum	AC	0.75	0.00	0.38	1	R

(*continued on the next page*)

Table 1. (*continued from the preceding page*)

Fungus	Type of record[a]	Recovery of fungi from lupine seeds (%)		Recovery (average) (%)	NIC[b]	Occurrence[c] remark
		From dishes with PDA	From dishes with wet filter paper			
F. poae	AB	2.00	1.50	1.75	4	L
F. semitectum	A	0.63	1.25	0.94	6	L
Mucor sp.	C	2.50	0.00	1.25	3	R
M. hiemalis	A	0.00	4.00	2.00	2	R
Penicillium sp.	B	1.00	0.75	0.88	7	M
P. citrinum	A	0.75	0.00	0.38	1	R
P. oxalicum	A	0.75	0.00	0.38	1	R
P. purpurogenum	AB	0.00	2.00	1.00	3	R
Phoma sp.	B	1.08	0.00	0.54	4	L
P. herbarum	A	0.00	2.00	1.00	2	R
P. sorghina	A	0.00	3.00	1.50	2	R
Rhizopus sp.	B	1.02	0.00	5.13	5	L
R. stolonifer	A	15.50	38.88	22.19	16	H
Sclerotium bataticola	A	0.00	2.75	1.38	3	R
Ulocladium atrum	A	0.00	2.50	1.25	2	R
Sterile mycelia (hyaline and dark)	B	0.88	1.13	1.01	7	M

[a] A, new record for lupine seeds; B, previously reported in the Sudan on other crops than lupine; C, new record for the Sudan.
[b] NIC: number of isolation cases, out of 26 replicates.
[c] H-high, more than 13 replicates, out of 26; M-moderate, between 7-13 replicates, out of 26; L- low, between 4-6 replicates out of 26; R- rare, less than 4 replicates, out of 26.

Table 2. Seed germination, fungal contamination, moisture content and aflatoxin presence (μg/kg) in 13 seed samples of lupine.

Sample Number	Germination (%)	Contamination by fungi (%)	Moisture content (%)	Presence/absence of aflatoxins
1	62.00	38.00	8.73	_
2	83.50	43.25	7.45	_
3	86.75	55.50	13.50	_
4	78.75	23.00	6.63	_
5	94.00	32.50	10.04	_
6	99.50	39.75	11.15	_
7	69.25	42.75	8.16	B_1 and B_2 (4.5 μg/kg)
8	85.75	36.50	7.20	_
9	92.50	22.25	12.34	_
10	66.00	12.75	5.42	_
11	90.00	19.25	9.64	_
12	70.00	59.75	8.04	B_1 and B_2 (6.5 μg/kg)
13	90.50	42.50	12.09	_

bean, cowpea, faba bean, lentil, pea and soybean, the genus *Aspergillus* was found to be the most common (Abdel-Hafez and Shoreit, 1986; Zohri and Abdel Gawad, 1992; El-Kady and Youssef, 1993; Moslem and Parvez, 1993). In the current study, the genus *Aspergillus* was also the predominant genus (10 species and 8 varieties, 42% of the total fungal colony count), followed by *Rhizopus*, *Fusarium*, and *Alternaria*. The remaining genera displayed very low levels of infection (16%).

In a previous study (Moslem and Parvez, 1993), it was reported that infection of legume seeds with fungal genera such as *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium*, *Curvularia*, *Drechslera*, *Ulocladium*, *Chaetomium*, *Mucor* and *Rhizopus* affected seed germinability to varying degrees. In the present study, the seeds of lupine were likewise highly contaminated with various species of all these genera (12.75-59.75%, average 35.21%) and showed much reduced seed germination (62.0-99.5%, average 82.15%).

A number of serious pathogens have been reported in lupine seeds with varying rates of infection (Werner 1991/92; Moslem and Parvez, 1993). In the current study, 5 species of *Alternaria* (*A. alternata* 1.75%, *A. brassicicola* 0.63%, *A. chlamydospora* 0.63%, *A. tenuis* 0.67%, *A. tenuissima* 1.63%), and *F. oxysporum* (0.38%) were isolated together with several pathogenic species from genera commonly known to be saprophytic genera (*Aspergillus, Chaetomium, Curvularia, Drechslera, Mucor, Penicillium, Rhizopus* and *Ulocladium*).

Pulses are liable to contamination with various mycotoxins, mainly aflatoxins (Habish, 1972; FAO, 1979; EL-Maraghy, 1989). In the present study, the common aflatoxin producers *A. flavus* var. *columnaris* (7.27%), *A. flavus* var. *flavus* (5.90%) and *A. parasiticus* (0.38%) were recovered from lupine seeds. The BGYF of 13 seed samples (Table 2) revealed that 2 samples contained aflatoxins. TLC of the chloroform extracts showed that these samples contained low concentrations of aflatoxins B_1 and B_2 (4.5-6.5 µg/kg seeds). It should be noted that TLC, the type of analysis performed, is not a very sensitive technique; moreover, lupine seeds were shown to be colonized by other potential toxigenic fungi as well. The possibility cannot therefore be excluded that other samples were contaminated by aflatoxins or other mycotoxins.

The study performed also found that the seeds of lupine may carry serious pathogenic fungi; the seeds used for sowing should therefore be treated chemically, stored properly. Moreover, as a safeguard to human health, all the seeds should be evaluated early for the presence of these toxic metabolites.

Acknowledgements

This research was supported by Prof. E.M.M. Abdel Bari of the University of Qatar. The facilities and space provided by the Department of Botany and the Computer Centre are highly appreciated. Thanks are due to Dr. A.H. Abdalla, Dr. S.A. Ibrahim, Dr. S. Eltigani, Dr. A.H.M. Ahmed , Miss. A.A. Behairi and Mrs. M.J.S. Dafaalla for their advice and encouragement.

Literature cited

Abdel-Hafez A.I.I. and A.A.M. Shoreit, 1986. Mycoflora of bean, lupine and pea seeds in Egypt. *Aswan Scientific and Technical Bulletin,* 7, 121-141.

Bothast R.J. and C.W. Hesseltine, 1975. Bright greenish-yellow fluorescence and aflatoxin in agricultural commodities. *Applied Microbiology*, 30, 337-338.

El-Kady I.A. and M.S. Youssef, 1993. Survey of mycoflora and mycotoxins in Egyptian soybean seeds. *Journal of Basic Microbiology*, 33(6), 371-378.

El-Maraghy S.S.M., 1989. The fungal flora and natural occurrence of mycotoxins of some leguminous varieties and hybrid seeds. *Bulletin of Faculty of Science*, *Assuit University*, 18, 63-75.

Elshafie A.E., 1986. Taxonomic studies on seed-borne fungi of the Sudan, II: Curvularia. *Sudan Journal of Science*, II, 51-70.

FAO, 1979. Perspective on mycotoxins. FAO and Nutrition Paper No. 13, FAO, Rome, Italy.

Gärber J. and M. Jahn, 1990. Investigation on the occurrence of seed-borne pathogens in legumes. *Nachrichtenblatt für den Pflanzenschutz in der DDR*, 44(3), 50-52.

Habish H.A., 1972. Aflatoxin in haricot bean and other pulses. *Experimental Agriculture*, 8, 135-137.

Hitokoto H., S. Morozumi, T. Wauke, S. Sakal, and H. Kurata, 1981. Fungal contamination and mycotoxin-producing potential of dried beans. *Mycopathologia*, 73, 33-38.

ISTA, 1966. International rules for seed testing. *Seed Science and Technology*, 4, 3-49.

Moslem M.A. and S. Parvez, 1993. Seed-borne fungi of *Lens esculentus*, *Hordeum vulgare* and *Triticum aestivum* from Saudi Arabia. *International Journal of Tropical Plant Diseases*, 11(1), 99-105.

Neergaard P., 1977. Seed Pathology. The Macmillian Press Ltd. London, UK, 3, 519-839.

Pate J.S., W. Williams and P. Farrington, 1985. Lupine (*Lupinus* spp.). *In:* Grain Legume Crops. (Summerfield R.J., Roberts E.H. eds). William Collins, London, UK, 699-746.

Singh K., J.C. Frisval, U. Thrane and S.M. Mathur, 1991. An illustrated manual on identification of some seedborne Aspergilli, Fusaria, Penicillia and their mycotoxins. Danish Government Institute of Seed Pathology for Developing Countries, Hellerup, Denmark, 133 pp.

Tarr S.A.J., 1963. A Supplementary List of Sudan Fungi and Plant Diseases. Mycological paper No. 85, Commonwealth Mycological Institute, Kew, Surrey, England.

Werner M., 1992. Pathogenicity of eight formae speciales of *Fusarium oxysporum* Schlecht in relation to different plant species. *Acta Mycologica*, 27(1), 127-136.

Zohri A.A. and K. Abdel Gawad, 1992. Studies on mycoflora and mycotoxins of cowpea cultivars. *Korean Journal of Mycology,* 20(3), 252-258.

Accepted for publication: March 3, 2000