

Seed-borne mycoflora of sesame seeds and their control using salt solution and seed dressing with Dithane M-45

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Abstract

Although seed is key in agricultural production, the seed health status of sesame seeds grown in Uganda is largely unknown. Seed health test of 30 seed samples from different parts of Uganda revealed the presence of 12 fungal species, namely, *Acremonium* sp., *Alternaria sesami*, *A. sesamicola*, *Bipolaris* sp., *Cercospora sesami*, *Corynespora cassiicola*, *Fusarium equiseti*, *F. moniliforme*, *F. pallidoroseum*, *Macrophomina phaseolina*, *Myrothesium roridum* and *Phoma* sp. High incidence, 0.3 – 68% of *A. sesamicola* and 0.3 – 29% of *C. sesami* were recorded in 29 and 30 seed samples, respectively. The rest of the pathogens occurred in moderate levels, 0.3 – 5% for *Acremonium* sp., *Corynespora cassiicola*, *A. sesami*, *F. equiseti*, *M. phaseolina*, *F. pallidoroseum*, *F. moniliforme* and *Phoma* sp. and in trace amounts, 0 – 0.3% for *Bipolaris* sp. and *M. roridum*. These pathogens caused seed rot, poor germination and seedling mortality. Two control measures; sorting by salt solution and seed-treatment with Dithane M-45 were used to separate healthy seeds from diseased ones, and kill seed-borne fungi, respectively. Both sunken and floated seeds had infected and healthy seeds in the samples tested indicating the use of salt solution, was not effective for obtaining healthy seeds in sesame. Seed treatment with Dithane M-45 killed all the mycoflora except *A. sesamicola* which was detected in 4 out of 14 samples but with a reduced incidence of 0 – 0.5% as opposed to 0 – 66% in the control. Thus, seed-dressing with Dithane M-45 is recommended for control of sesame seed-borne pathogens.

Key words: Fungi, seed health testing, seed treatment, sorting

Introduction

Sesame (*Sesamum indicum* Linn.) is one of the most important oil seed crops in the sub-Saharan Africa. The oil is used for a variety of purposes, namely, cooking, soap manufacture, food and medicine as well as an adulterant for olive oil. Sesame oil is also used as an important ingredient in most cosmetic industries. In addition, seeds are added to cookies and other baked goods and made into candy. Sesame meal is an excellent, high-protein source (34 to 50%) for poultry and livestock feeding (Oplinger *et al.*, 1990).

In Uganda, the yield of sesame is 300 – 430 kg ha⁻¹ compared to the potential yield of 2250 kg ha⁻¹ (FAO, 2000). Low quality seeds have been reported among the most important constraint to sesame production (Mathur and Kabeere, 1975; Singh *et al.*, 1980; Yu *et al.*, 1982). Work by Mathur and Kabeere (1975) revealed high incidence of seed-borne pathogens such as *Alternaria sesamicola*, *Cercospora sesami*, *Corynespora cassiicola*, *Fusarium* spp. and *Macrophomina phaseolina*, with *A. sesamicola* and *C. sesami* being most predominant and causing heavy seedling mortality and seed rot.

In general, however, limited research has been conducted on seed quality and seed health of sesame seeds from Uganda. Also, little work on control of seed-borne pathogens of sesame has been done. Yet according to Venter (2000) the first step towards the attainment of maximum crop yield is the use of

high quality seeds. The objectives of this study therefore, were to determine the health status and quality of sesame seeds produced in Uganda, and to identify plausible control strategy against seed-borne fungal pathogens of sesame.

Materials and methods

Thirty seed samples collected from various parts of Uganda during the 2000/2001 crop season, comprised of varieties locally grown by farmers as well as those released by the Natural Research Organisations. Seed samples were stored at 25°C and 28°C at Makerere University before transferring them to Denmark. In Denmark, the seeds were stored at 5°C at the Danish Government Institute of Seed Pathology for Developing Countries (DGISP) for 3 weeks before the start of experiments. All the seed samples were assigned accession numbers and working samples of approximately 7g, were drawn from each accession following the International Rules for Seed Testing (ISTA, 1999) and seed health, germination and control of sesame seed-borne fungi investigated.

Seed health testing

Seed samples of 30 Ugandan cultivars were assayed for seed health. Detection of seed-borne fungi was done by blotter method following procedures outlined by ISTA (1999) and Mathur and Kongsdal (2000). Four hundred seeds randomly selected from each working sample of 7 grams were plated on three pieces of well water-soaked blotters in plastic petri-dishes (9 cm in diameter). Each petri-dish contained 25 seeds and were incubated at 20±2 °C for 7 days under alternating cycles of 12 hours darkness and near ultraviolet (NUV) light (Philips, TLD 36 W/08). After incubation, seeds were observed for the growth of fungi under a stereo-binocular microscope. The fungal species were identified by their "habit characters" and later confirmed by morphological characteristics of the fruiting bodies, conidia and spores using a compound microscope (Mathur and Kongsdal, 2000). Percentage incidence of pathogenic fungi was computed and recorded.

Germination test

The seed samples were also assayed for germination capacity using Top of Paper (TP) method (ISTA, 1999). Four hundred seeds in replicates of 100 were taken at random from each working sample. Subsequently, each replicate sample was divided into sub-samples of 50 seeds each and spaced uniformly and adequately apart on three pieces of well water-soaked filter paper in germination boxes (16x10 cm). The germination boxes with their contents were incubated in a climate room with day and night temperatures of 26°C and 24°C, respectively for six days. The number of normal and abnormal seedlings, ungerminated (dead) seeds was recorded.

Control of seed-borne fungi by salt sorting

Five sesame seed samples randomly selected from accessions with germination percentage >75%, the minimum recommended by the Uganda Seed Project (USP) (ST, 1994), were used in the experiment to separate fungal infected seeds from healthy seeds using salt solution. Four hundred seeds were taken from each sample and separated by density using 0%, 2.5% and 5% salt concentrations. At each level of salt concentration, sunken seeds and floated seeds were separated and assayed for seed health and germination. The number of seeds tested at each level of salt concentration depended on the quantity of sunken and floated seeds.

Control of seed-borne fungi using Dithane M-45

Fourteen sesame seed samples treated with Dithane M-45 at a rate of 2.0g kg⁻¹ were assayed for both germination and seed health. These levels of infection were considered, namely, high (= 50%), moderate (=20 & <50%) and low infections (<20%). For each category the test samples were selected randomly. The seeds were dressed with the fungicide and kept for about 24 hours after which the seed health and germination assays were conducted.

Data analysis

All the data collected in the laboratory were subjected to analysis of variance (ANOVA) using Sigmastat – 2.0 computer package. Means separation were performed using Tukey test at P = 0.05.

Results*Seed health*

Twelve fungal species belonging to 9 genera were identified, but the level of seed infection varied among the samples (Table 1). The most predominant pathogens detected were *Alternaria sesamicola* and *Cercospora sesami*. The pathogens with moderate occurrence and low incidence were *Fusarium moniliforme*, *Phoma* sp., *Acremonium* sp., *F. equiseti*, *Corynespora cassicola*, *Macrophomina*

Table 1. Incidence (%) of different fungal species detected by blotter method in 30 sesame seed samples from Uganda¹.

DGISP Accession No.	Fungal species ²											
	Acs	As	Asc	Bs	Cs	Crc	Fe	Fm	Fp	Mp	Myr	Ps
46494	0.5	0	40.3	0	25.8	0	0	1.8	0	0.3	0	1.8
46498	0	0	60.8	0	5.8	0	0	0.5	0	2	0	1
46507	0	0	36	0	26	0	0	2.8	0.5	0.5	0	0.3
46511	0	0.5	23	0	17.3	0.3	0	1.5	0	0	0	0.5
46512	0	0	56.5	0	7.3	0.5	0	3	0.3	0	0	1.5
46513	0	0	28.3	0	24.5	0	0	2.3	0	0.3	0	0
46516	0	0	0	0	16.3	0	0	0	0	0	0	0
46517	0	1	68.3	0	20.3	0	2.3	0.3	2.5	0	0	3.8
46520	0	0	3.8	0	4.5	0	0	0	0	0	0	0
46522	0	0.3	53	0	4.3	0.5	0	0	0.3	0	0	1.8
46530	0	0.5	29.3	0	21	0	0	1.5	0.3	0	0	0.3
46532	0	0.5	34	0	14.5	0	0.3	2.3	0	0.3	0	1
46533	0	0	26	0	16	0	0.3	2.5	0	0	0	0.8
46534	0.3	0	20.3	0	16	0.5	0.3	0.8	0.8	0	0	1
46539	0	0	43.3	0	19.3	0	0	1.5	0.3	0.3	0	2.8
46540	0	0.3	23.5	0	16.3	0	0	0.8	0.3	0.5	0	0.8
46541	0	0	58.5	0	11	1	0	4.5	2.3	0	0	1.5
46542	0	0.3	57.8	0.3	11.5	0	0.3	0.5	0.3	0.8	0	0.3
46543	0	0	36.3	0	16	0.5	0	2	0.3	1	0	0
46544	0	0	0.8	0	5.5	0	0	0.3	0	0	0	0.5
46545	0	0	1.3	0	6.8	0	0.3	0	0.3	0	0	4.3
46546	0	0	0.5	0	1.8	0	0	0.3	0	0	0	0.3
46547	0	0	0.3	0	0.3	0	0	0	0	0	0	0
46548	1.5	0.5	47.8	0	16.8	0	0	0.3	0.3	0	0	0.8
46549	0	0	15.3	0	28.5	0.3	0	2	0.5	0	0	0.3
46550	0	0	24	0	17.8	0.5	0	3.5	0	0	0	2
46552	0.3	0.3	22	0	14.8	0.3	0	3.8	0	0	0.3	1.5
46555	0	0.3	50	0	24	0	0	0.5	0	0	0	0.5
46556	0	0	58.3	0	3.8	0	0	0.8	0	0	0	0.8
46558	0	0	65.5	0	23.8	0	0	0.3	0	0	0	0.3

¹Four hundred seeds were tested as recommended by ISTA (1999).

²Acs = *Acremonium* sp., As = *Alternaria sesami*, Asc = *A. sesamicola*, Bs = *Bipolaris* sp., Cs = *Cercospora sesami*, Crc = *Corynespora cassicola*, Fe = *Fusarium equiseti*, Fm = *F. moniliforme*, Fp = *F. Pallidoroseum*, Mp = *Macrophomina phaseolina*, My = *Myrothecium roridum*, Ps = *Phoma* sp.

phaseolina, *A. sesami* and *F. pallidoroseum*. Those occurring in only one sample and with very low incidence were *Bipolaris* sp. and *Myrothesium roridum*. Besides the pathogenic fungal species, saprophytic fungi were also detected, namely, *A. alternata*, *Aspergillus* spp., *Cladosporium* sp., *Penicillium* sp. and *Rhizopus* sp (Fig. 1).

Germination test

Fourteen of the 30 sesame cultivars had germination percent of = 75%, which is within the threshold set by the Uganda Seed Project (ST, 1994). The cultivars which had lower germination percentage had seedlings associated with various defects including decayed roots, hypocotyls and cotyledons (Fig. 2).

Control of seed-borne fungi by salt sorting

Seed health test of both sunken and floated seeds revealed that *Alternaria sesamicola* and *Cercospora sesami* were still the dominant pathogens. *C. sesami* was present in all the samples (100%) and *A. sesamicola* was detected in 80% of the samples tested at all levels of salt concentration, including the control. Unlike the sunken seeds, *Macrophomina phaseolina* was not detected in all the salt floated seeds. The range of infection in floating seeds (FS) was slightly higher for most of the pathogens than in sunken seeds. For instance, incidence of *A. sesamicola* ranged from 0 – 72% in floating seeds compared to 0 – 64% in sunken seeds at the same salt concentration level (0%) (Table 2). Both sunken and floated seeds at different salt concentrations achieved < 75% germination (Table 3), the minimum recommended by the Uganda Seed Project (ST, 1994).

Control of sesame seed-borne fungi by Dithane M-45

Seed treatment with Dithane M-45 eliminated all seed-borne pathogens, as detected by the blotter test, except *Alternaria sesamicola* which was still detected in 29% of the samples assayed (Table 4). Even then, the range of seed infection by *A. sesamicola* in the Dithane M-45 treated seeds reduced drastically to 0 – 0.5% compared to 0 – 66% in untreated seeds (Table 4).

Twelve of the 14 accessions showed increase in germination percentage as a result of seed treatment with Dithane M-45 as compared to the untreated ones. Unlike the untreated seeds, the number of abnormal seedlings due to primary infection was greatly reduced in the treated seed samples. For instance, brown discolouration and decay of roots seen on seedlings from the untreated seeds were not observed on seedlings treated with Dithane M-45 (Fig. 3). No improvement was detected in two accessions (46520 and 46544) (Table 5).

Discussion

Several seed-borne fungal pathogens were identified in this study. These included *Alternaria sesami*, *A. sesamicola*, *Bipolaris* sp., *Cercospora sesami*, *Corynespora cassiicola*, *Fusarium equiseti*, *F. moniliforme*, *F. pallidoroseum*, *Macrophomina phaseolina*, *Myrothesium roridum* and *Phoma* sp. These results therefore correlate with earlier reports that sesame seeds are heavily infected by a number of seed-borne pathogens (Mathur and Kabbere, 1975; Singh *et al.*, 1980, 1983; Yu *et al.*, 1982; Richardson, 1990; Poswal and Misari, 1993). *Alternaria sesamicola* which had the highest incidence of 68% is known to cause *Alternaria* leaf spot and blight (Singh *et al.*, 1980, 1983; Yu *et al.*, 1982). *Cercospora sesami* which causes *Cercospora* leaf spot occurred on all the seeds assayed although with varying incidence. Heavily infected seeds did not germinate an indication that the pathogen was responsible for failure in germination. The disease has been reported by Poswal and Misari (1993) to spread from leaf to leaf and plant to plant very fast once established. There is a need therefore to eradicate these two predominant pathogens. However, other pathogens detected in moderated to trace amounts should not be ignored because they may pose a threat to sesame production in the long run.

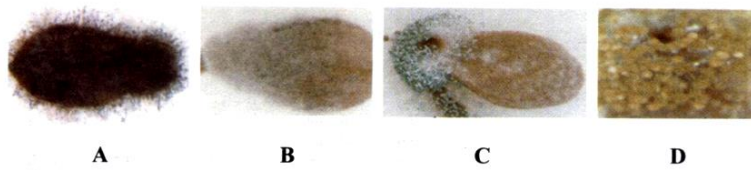


Figure 1. Sesame seeds showing different fungal growth which developed during incubation for seed health test. A: *Alternaria sesamicola*, B: *Cercospora sesami*, C: *Penicillium* sp., D: *Aspergillus flavus*.



Figure 2. Defects shown by sesame seedlings as a result of fungal infection in the germination test. A: Cotyledons necrotic, B: Whole seedling decayed.



Figure 3. Germination of untreated and Dithane M-45 treated sesame seeds. Seedlings from untreated seed (left) show decay and discoloration of roots due to primary infection; treated seed have clear appearance (right).

Table 2. Occurrence (%) of different fungal species on sesame seed detected in salt medium.

Fungal species ¹	Samples infected (%) ²				Range of infection (%)			
	0	2.5	5	Ufs	0	2.5	5	Ufs
Sunken seeds								
As	20	20	20	20	0-2	0-0.4	0-0.4	0-1
Asc	80	80	80	80	0-64	0-48	0-57	0-68
Cs	100	100	100	100	12-25	15-22	12-23	7-26
Fe	20	0	20	20	0-4	0	0-0.4	0-2
Fm	60	60	40	80	0-1	0-1	0-1	0-3
Fp	40	40	20	60	0-3	0-2	0-0.4	0-3
Mp	20	20	20	20	0-0.3	0-0.4	0-2	0-1
Ps	80	80	60	80	0-3	0-3	0-0.3	0-4
Floated seeds								
As	20	20	0	20	0-2	0-2	0	0-1
Asc	80	80	80	80	0-72	0-52	0-50	0-68
Cs	100	100	100	100	12-28	13-25	17-25	7-26
Fe	20	20	20	20	0-2	0-1	0-1	0-2
Fm	20	20	40	80	0-2	0-1	0-1	0-3
Fp	40	40	40	60	0-3	0-1	0-1	0-2
Mp	0	0	0	20	0	0	0	0-1
Ps	60	60	80	80	0-9	0-6	0-3	0-4

¹As = *Alternaria sesami*, Asc = *Alternaria sesamicola*, Cs = *Cercospora sesami*, Fe = *Fusarium equiseti*, Fm = *F. moniliforme*, Fp = *F. pallidoroseum*, Mp = *Macrophomina phaseolina*, Ps = *Phoma* sp.

²0 = water without salt, 2.5 = 2.5% salt solution, 5 = 5% salt solution Ufs = Unfloated seeds.

Table 3. Germination using top of paper (TP) method of sunken seeds (SS) and floated seeds (FS) in salt medium solution^{1, 2}.

DGISP Acc. No.	Unfloated seed ³	0% salt concentration	2.5% salt concentration	5% salt concentration
Sunken seeds				
46507	71a	73a	70a	73a
46512	69a	60b	67a	66a
46516	64a	54b	65a	66a
46517	59a	59a	61ab	64b
46558	57a	66b	59a	72c
Floated seeds				
46507	71a	59b	66c	72a
46512	69a	52b	52b	64c
46516	64a	51b	58c	61ac
46517	59a	36b	37b	44c
46558	57a	39b	49c	60a

¹Pooled data for 2 tests.

²Means in the same row followed by the same letter are not significantly different at P = 0.05

³Unfloated seeds: seeds not separated in salt solution and used as a control.

Germination tests revealed that sesame seeds produced in Uganda have low germination capacity, less than 50% of the seed samples tested meeting the national standards of = 75% germination. A key factor which reduced germination percentage was infection by pathogenic fungi. These fungi affected roots, hypocotyls and cotyledons. Infected roots were decayed, hypocotyls necrotic and cotyledons decayed as a result of primary infection. These results correlate with earlier findings by Mathur and Kabeere (1975). Plants which were raised from infected seedlings remained stunted with spots on all the leaves and eventually collapsed after 40 – 45 days of growth. However, the tolerant plants developed severe spots and blight on leaves and pods.

Table 4. Frequency of occurrence (%) of different fungal species on untreated and Dithane M-45 treated sesame seed samples as detected by blotter test.

Fungal species	Samples infected (%)		Range of infection (%)	
	Untreated seed	Treated seed	Untreated seed	Treated seed
<i>Alternaria sesami</i>	14	0	0 – 1	0
<i>A. sesamicola</i>	93	29	0 – 66	0 – 0.5
<i>Cercospora sesami</i>	100	0	3 – 25	0
<i>Corynespora cassiicola</i>	14	0	0 – 1	0
<i>Fusarium equiseti</i>	14	0	0 – 3	0
<i>F. moniliforme</i>	79	0	0 – 6	0
<i>F. pallidoroseum</i>	38	0	0 – 3	0
<i>Macrophomina phaseolina</i>	14	0	0	0
<i>Phoma</i> sp.	79	0	0 – 3	0

Table 5. Germination of untreated and Dithane M-45 treated sesame seed samples using top of paper (TP) method¹.

DGISP Accession No.	Germination percentage ²					
	Test 1		Test 2		Mean ³	
	Untreated seed	Treated seed	Untreated seed	Treated seed	Untreated seed	Treated seed
46494	84	94	80	93	82a	94b
46498	64	83	62	83	63a	83b
46507	70	87	73	87	72a	87b
48512	73	84	70	83	72a	84b
46516	61	70	66	74	64a	72b
46517	58	82	58	81	58a	82b
46558	52	88	60	90	56a	89b
46522	74	88	73	86	74a	87b
46533	65	85	69	89	67a	87b
46548	77	88	78	90	78a	89b
46556	76	91	78	91	77a	91b
46541	84	90	82	90	83a	90b
46544	62	63	62	64	62a	64a
46520	52	54	46	51	49a	53a
Mean	68	82	68	82	68	82

¹Top of paper (TP) method: seeds are germinated on top of one or more layers of paper which are placed into transparent boxes (ISTA, 1999).

²Normal seedlings: seedlings that show the potential for continued development into satisfactory plants when grown in good quality soil and under favourable conditions of moisture, temperature and light (ISTA, 1999).

³Means in the same row followed by the same letter are not significantly different at P = 0.05.

Salt sorted seeds

The presence of fungal species in the sunken seeds thought to be free of infected seeds is an indication that salt solution did not separate healthy from infected seeds. The number of samples infected and the range of infection by various pathogens was similar to that of the control (unfloated) seeds. This implies that both sunken and floated seeds had more or less similar numbers of diseased and healthy seeds. Similarly in the germination test, of the 3 salt concentration levels compared, none of them produced seeds with the minimum germination percentage of = 75% recommended by the Uganda Seed Project, suggesting that salt solution did not effectively separate diseased and healthy seeds. This was confirmed when most of the floated seeds presumed to be diseased were found healthy and vice-versa. However, this control measure has been reported to work with rice (Mabagala, 2001), eggplant and tomato (Quazi, 2001).

Dithane M-45 treated seeds

Results of the blotter test showed that seeds treated with Dithane M-45 were free from seed-borne pathogens as opposed to untreated seeds. Similar results were obtained in germination where apart from increased germination, there was significant reduction in number of abnormal seedlings resulting from primary infection. The results correlate with earlier reports that seed treatment with various fungicides killed seed-borne fungi and improved seed germination (Kumar and Agarwal, 1998; Jagadeesh and Lokesh, 1999; Vasundhara and Gowda, 1999). However, it is not clear whether all the seed-borne inoculum was killed since Dithane M-45 is a contact fungicide, and probably acted on only the superficial fungi.

This study revealed that Dithane M-45 treated sesame seeds had better germination and less mycoflora infection compared to untreated seeds. Since these results were only based on laboratory and greenhouse work, we recommend that field experiments be conducted for purposes of verification under field conditions and also to establish seed to seedling transmission of the seed-borne pathogens. Attention should be given to the two common pathogens, *A. sesamicola* and *C. sesami*.

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