FUNGI OF STORED *KHAYA ANTHOTECA* SEEDS AND THEIR EFFECT ON GERMINATION

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MITTAL, R. K., MATHUR, S. B. & THOMSEN, K. 2003. Fungi of stored *Khaya anthoteca* seeds and their effect on germination. Twenty-one fungi belonging to 18 genera were identified on seeds of *Khaya anthoteca* stored under 8.2% moisture content for 12 months at -18, 5 and 15 °C. *Botryodiplodia theobromae, Fusarium solani, F. pallidoroseum* and *Macrophomina phaseolina* were the predominant species. High occurrence of *Rhizoctonia solani, Fusarium* sp., *Pyrenochaeta* sp. and *Cylindrocladium* sp. on the ungerminated seeds could have caused decay of seeds thus resulting in erratic germination results. Seeds stored at -18 or 5 °C had a higher occurrence of fungi and showed significantly lower germination than those stored at 15 °C, indicating the detrimental effect of fungi under unfavourable storage environments. *Fusarium solani* and *F. pallidoroseum* were identified on a large number of ungerminated seeds, suggesting their detrimental effects on seeds during storage.

Key words: Seed-borne - occurrence - storage - temperatures - health - moisture content

MITTAL, R. K., MATHUR, S. B. & THOMSEN, K. 2003. Kulat pada biji benih *Khaya anthoteca* dalam simpanan dan kesan kulat terhadap percambahan. Dua puluh satu kulat daripada 18 genus dikenal pasti pada biji benih *Khaya anthoteca* yang disimpan pada kandungan lembapan 8.2% selama 12 bulan pada suhu -18 °C, 5 °C dan 15 °C. *Botryodiplodia theobromae, Fusarium solani, F. pallidoroseum* dan *Macrophomina phaseolina* merupakan spesies kulat yang utama. Kehadiran *Rhizoctonia solani, Fusarium* sp., *Pyrenochaeta* sp. dan *Cylindrocladium* sp. yang kerap pada biji benih yang tak bercambah mungkin menyebabkan pereputan biji benih. Ini memberi keputusan percambahan yang tidak menentu. Biji benih yang disimpan pada -18 °C dan 5 °C mempunyai kekerapan kulat yang lebih tinggi dan menunjukkan percambahan yang kurang berbanding biji benih yang disimpan pada 15 °C. Ini menunjukkan kesan kulat yang menjejaskan di bawah keadaan simpanan yang tidak menggalakkan. *Fusarium solani* dan *F. pallidoroseum* dikenal pasti pada banyak biji benih yang tak bercambah. Ini mencadangkan kesan kulat yang menjejaskan ke atas biji benih dalam simpanan.

Introduction

Khaya anthoteca, commonly known as African mahogany, is an important tree species grown for timber in the tropical regions. Work on storage requirements of the species is in progress under the Project on Handling and Storage of Recalcitrant and Intermediate Tropical Tree Species, of the International Plant Genetic Resource Institute, Rome, Italy and Danida Forest Seed Centre (DFSC), Humlebæk, Denmark. In the germination tests conducted on seeds stored for 12 months at the DFSC, many seeds failed to germinate and great variation in germination was observed. Fungal growth was also visible on many ungerminated seeds. This paper discusses the studies on such ungerminated seeds and reports on the fungi detected on stored seeds and their possible effects on seed health.

Materials and methods

The fruits were collected on 22–26 May 1997 in Tanzania and the seeds, which were extracted soon after collection, were received at the DFSC on 4 June 1997. The initial moisture content of the whole seeds and germination were 11.4 and 85% respectively in Tanzania, and 8.2 and 52% at the DFSC. No information on seed-borne fungi of the seeds after their collection was available. After 12 months of storage at -18, 5 and 15 °C, these seeds (100 seeds × 4 replicates) were tested for germination in vermiculite in propylene boxes at the DFSC where a large variation in germination between the replicates was observed (Thomsen 1998), and many seeds which failed to germinate after four weeks showed fungal growth. Boxes containing such ungerminated seeds were carefully brought to the Danish Government Institute of Seed Pathology, Copenhagen, Denmark (DGISP) for further investigations on seed health. Seed-borne fungi of seeds which were stored at three different temperatures (-18, 5 and 15 °C) were also detected using standard blotter method (ISTA 1993). The germination of such seeds and the possible effects of seed-borne fungi on germination and seedling quality were also studied. Seeds with about 5 mm or more growth of radicle from the seed coat were considered germinated.

Studies on ungerminated seeds

Seventy-five ungerminated seeds (47 of -18 °C and 28 of 5 °C storage) were cut open to see whether they were empty or filled. Fungi growing on them were observed microscopically. The other 18 ungerminated seeds (combined from both the storage treatments) were treated with 1% sodium hypochlorite for 3 min followed by rinsing twice with deionised water and tested on moist blotters to ascertain whether the fungus was internally located. Root tips of 19 germinants which showed arrested growth and root tip blighting were excised (5 mm length), treated similarly with sodium hypochlorite and plated on potato dextrose agar to detect if associated fungi could have caused the symptoms.

Detection of fungi in stored seeds

Fifty seeds, each of the lot stored for 12 months at the three different temperatures, were tested using the blotter method after treatment with 1% sodium hypochlorite for 3 min followed by rinsing twice with deionised water. To avoid seed-to-seed contamination, seeds were tested individually in 30-mm pre-sterilised disposable Petri dishes. Observations were made after seven days of incubation at 21 °C with 12 hours NUV light. The number of germinated seeds was also counted to find any relation between fungal occurrence and seed germination.

Growing-on test

Fifty seeds, each of the lot stored for 12 months at the three different temperatures, were treated with 1% sodium hypochlorite for 3 min followed by rinsing twice with deionised water, and tested for quantity and quality of seed germination in moist vermiculite in a growth room at 26 °C with 12 h photoperiod. To avoid seed-to-seed contamination, one seed was sown per plastic pot (65 × 65 mm size). Observations were made every seven days. Seeds that did not germinate after four weeks were removed, treated with 1% sodium hypochlorite for 3 min followed by rinsing twice with deionised water, and tested individually on moist blotter in pre-sterilised disposable Petri dishes. After seven days of incubation at 21 °C with 12 hours NUV light, the fungi on seeds were studied. Observations on seedling health were conducted up to eight weeks after sowing.

Results and discussion

Studies on ungerminated seeds

All the 47 ungerminated seeds from -18 °C storage were internally decayed. *Fusarium* sp. colonised all four randomly selected seeds examined directly under the microscope. Of the 28 ungerminated seeds from 5 °C storage, 27 were internally decayed with only one apparently healthy. *Fusarium* sp. was present on four out of five seeds picked up randomly, and *Rhizoctonia solani* on the remaining one seed.

The following fungi were observed on the 18 ungerminated seeds tested for seed health on moist blotter: *R. solani* on 83.4%, *Fusarium* sp. on 43.8%, *Pyrenochaeta* sp. on 57.5%, *Cylindrocladium* sp. on 28.5% and *Curvularia* sp. on 6.3%.

Only 10% of the root tips of 19 germinants which showed arrested growth and root tip blighting showed growth of *Fusarium* sp.

Detection of fungi in stored seeds

Twenty-one fungi belonging to 18 genera were identified on the seeds (Table 1). *Botryodiplodia theobromae, Fusarium solani, F. pallidoroseum* and *Macrophomina phaseolina* were the predominant species. *Rhizoctonia solani,* which infected a large number

of ungerminated seeds, was observed on only 4% seeds which were stored at 5 °C and on 10% seeds stored at -18 °C. During the moist blotter test, only 2, 14 and 54% seeds germinated from the lots stored at -18, 5 and 15 °C respectively.

Most of the observed fungi are known seed pathogens. They have been recorded on seeds of several tree species (Mittal *et al.* 1990). Species of *Fusarium, Aspergillus* and *Curvularia* have also been reported on seeds of *K. senegalensis* from Nigeria (Adegeye *et al.* 1985). *Fusarium* spp. and *Pestalotia* sp. have been identified on *K. grandifolia* seeds of Ghana (Gyimah 1987). Abdel Latif *et al.* (1991) isolated *F. oxysporum* and *Thanatephorus cucumeris* from the damped-off seedlings of *K. senegalensis.*

Depending on the storage conditions, *F. solani, F. pallidoroseum, B. theobromae, M. phaseolina* and *R. solani* were observed on a greater number of seeds and they had proliferated more extensively than the other fungi. Generally, these species were more apparent on seeds which were stored at -18 and 5 °C than on seeds stored at 15 °C (Table 1). Fungal infection was maximum on seeds stored at -18 °C, then on seeds stored at 5 °C and the least on seeds stored at 15 °C. Seed germination (54%) was best in seeds stored at 15 °C, followed by seeds stored at 5 °C and the minimum in seeds stored at -18 °C. This suggests that these fungi played a role in deterioration of seeds during storage. The results also indicate the chilling sensitivity of seeds, leading to tissue damage which would greatly facilitate fungal proliferation.

Table 1 Fungi on *Khaya anthoteca* seeds after storage for 12 months at -18, 5 and 15 °C

Fungus	Occurrence (%)		
	-18 °C	5 °C	15 °C
Alternaria alternata	4	-	-
Aspergillus niger	-	-	6
Bipolaris sp.	-	2	-
Botryodiplodia theobromae	24	6	14
Chaetomium globosum	2	4	4
Cladosporium cladosporoides	6	2	2
Curvularia pallescens	4	2	6
Curvularia eragrostidis	-	2	-
Cylindrocladium sp.	4	-	-
Fusarium equiseti	2	-	-
Fusarium pallidoroseum	20	14	4
Fusarium solani	40	18	8
Macrophomina phaseolina	4	24	2
Melanospora zamiae	2	-	-
Myrothecium roridum	4	-	-
Penicillium sp.	-	-	12
Pestalotia sp.	4	6	-
Phomopsis sp.	2	6	-
Rhizoctonia solani	10	4	-
Trichoderma viride	2	-	-
Ulocladium oudemansii	2	2	-

Growing-on test

After three weeks, 2, 8 and 80% of the seeds which were stored at -18, 5 and 15 °C respectively germinated. This represents a marked improvement for only those seeds stored at 15 °C, over the 54% germination in the moist blotter. No further germination took place the following week. No seedling showed symptom of any disease after eight weeks of sowing.

Sixteen fungal species belonging to 14 genera were identified on ungerminated seeds (Table 2). *Fusarium solani* infected a large number of seeds. It is an important pathogen and causes a number of diseases including root rot and damping-off of seedlings and decay of seeds. Interestingly, although *Pyrenochaeta* sp. has been observed on ungerminated seeds tested at the DFSC as well as in the present growing-on test, it was not observed on the seeds when studied directly for associated mycoflora. High incidence of *M. phaseolina* was observed in seeds in the blotter test but the fungus was not seen on ungerminated seeds. It is difficult to explain this. One possible reason could be the competition between fungal species on different testing media that allowed particular fungi to grow.

Table 2 Fungi recorded on ungerminated seeds collected from the growing-on test

Fungus	Occurrence (%)[a]		
	-18 °C	5 °C	15 °C
Alternaria alternata	2	2	10
Bipolaris sp.	2	-	-
Botryodiplodia theobromae	10	18	10
Chaetomium globosum	-	9	-
Curvularia pallescens	-	4	-
Curvularia eragrostidis	-	-	10
Cylindrocladium sp.	8	4	10
Fusarium pallidoroseum	12	36	-
Fusarium solani	73	33	30
Gliocladium roseum	6	-	-
Myrothecium roridum	8	11	-
Pereconia sp.	2	-	-
Phomopsis sp.	12	4	-
Pyrenochaeta sp.	12	15	30
Rhizoctonia solani	10	2	20
Ulocladium oudemansii	4	2	-

[a]At -18 °C, data were based on 49 ungerminated seeds, at 5 °C on 46 ungerminated seeds and at 15 °C on 10 ungerminated seeds

It is, however, difficult to ascertain whether the fungi invaded the seeds, first resulting in decreased viability or germinability, or the seeds were first weakened by low temperature which facilitated fungal growth. Berjak (1996) reported that the processes of both fungal deterioration and seed debilitation in adverse storage