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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (3): 321-331, 2008
ISSN 1028-8880

Importance of Seed-Borne Fungi of Sorghum and Pearl Millet in Burkina Faso and Their Control Using Plant Extracts

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Abstract: Seed-borne fungi of sorghum and pearl millet in Burkina Faso were surveyed. A total of 188 seed samples from various locations, collected in 1989 (42) and 2002 (146), were tested, using the blotter, dry inspection and washing methods. Infection experiments were carried out with the major fungi recorded on each crop by the blotter test. Six essential oils of plants were investigated for their inhibitory activity against eight pathogenic fungi. Thirty four and 27 fungal species were found in seed samples of sorghum and pearl millet, respectively. *Phoma* sp. and *Fusarium moniliforme* infected 95 to 100% of the seed samples of both sorghum and pearl millet. *Sphacelotheca sorghi* and *Tolyposporium ehrenbergii* were encountered in respectively, 75 and 33% of seed samples of sorghum. *T. penicillariae*, *Sclerospora graminicola* and *Claviceps fusiformis* were present in 88, 41 and 32% of seed samples of pearl millet, respectively. Seeds inoculated with *Acremonium strictum*, *Curvularia oryzae*, *F. equiseti*, *F. moniliforme* and *F. subglutinans* and sown in sterilized soil, showed considerable mortality of the seedlings. Three essential oils inhibited *in vitro* the mycelial growth of all the fungi used by 85 to 100% and reduced significantly sorghum and pearl millet seed infection rates of *Phoma* sp., *Fusarium* sp., *Curvularia* sp., *Colletotrichum graminicola* and *Exserohilum* sp. Presence of many pathogenic fungi in considerable number of seed samples indicates the need of field surveys for these and other pathogens. Development of plant extracts for the control of seed-borne pathogens and public awareness on seed-borne diseases management measures for maintaining quality seed should be increased.

Key words: Sorghum, pearl millet, seed-borne fungi, pathogenicity, plant extracts, biological control

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) and pearl millet (*Pennisetum glaucum* (L.) R. Br.) are the two major cereal of Burkina Faso. Annually grown on 2,641,089 hectares, representing 88% of the cereal areas, these two crops produced 2,100,046 tons of grain, corresponding to 79% of the total national cereal production (DSA/MAHRH, 2005). Sorghum and pearl millet are of vital importance because 70 and 95% of their respective production are consumed by populations in the main production regions (FAO and ICRISAT, 1997). Despite the socio-economic importance of these crops, their productivity has remained very low due to various constraints. The average yields recorded during the last ten years are 830 kg ha^{-1} for sorghum and 670 kg ha^{-1} for pearl millet (DSA/MAHRH, 2005; FAO, 2006). These yields are by far low from the world yields of sorghum and pearl millet which are 1332 and 816 kg ha^{-1}, respectively (FAO, 2006). Amongst the biotic constraints to sorghum and pearl millet production in Burkina Faso, seed-borne fungal diseases are of great importance (Neya and Kaboré, 1987; Séréme, 1995; Mathur and Manandhar, 2003). In Burkina Faso, sorghum and pearl millet seeds used by farmers are mainly from their own-saved seed lots; the only criterion considered by the farmers when selecting seeds is the physical appearance of the grain. Earlier investigations on sorghum seeds produced in

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different research stations in Burkina Faso (Kamboinsé, Farako-bâ, Kouaré, Di, Niangoloko) and two localities (Nakamtinga, Boni), revealed that these seeds were infected by important fungal pathogens such as *Colletotrichum graminicola* (Ces.) Wilson, the causal agent of anthracnose, *Gloeocercospora sorghi* (zonate leaf spots), *Phoma* sp. and *Fusarium* sp. (seedlings blights) (Kaboré et Couture, 1983; SOMIMA, 1994), but very little information is available on the occurrence of fungi on farmers'own saved seeds from various producing places. Therefore, a country-wide survey is necessary to identify the kind and amount of mycoflora associated with sorghum and pearl millet seeds in Burkina Faso and to assess their distribution in different ecological zones and their significance on seed and plant health. Breeding for genetic resistance and use of chemicals have been the most effective strategies recommended to control sorghum and pearl millet diseases (DPVC, 1995). But, improved varieties and chemicals are not always available or are often costly for the small-scale farmers; and the use of natural products as alternatives for synthetic pesticides is still limited. Furthermore, a repetitive use of synthetic pesticides can induce genetic resistance in target organisms, as resistance of *Ustillago nuda* (wheat smut) to carboxine, previously reported by Leroux (1986, 1987). In this context, development of simple and eco-friendly seed-borne diseases management methods is necessary to improve the quality of seed in general and farmers saved-seed in particular. In a recent study, Somda *et al.* (2007) demonstrated the efficacy of aqueous extracts of *Cymbopogon citratus* (DC) Stapf. for controlling sorghum seed infections by *C. graminicola* and *P. sorghina*. Many studies carried out in Burkina Faso underlined insecticidal properties of some plant species including *Cymbopogon*, *Hyptis* and *Ocimum* (Koumaglo *et al*., 1998; Djibo, 2000; Nébié, 2006). But information related to the role of such local plants in controlling crop pathogen agents is still limited.

The objectives of the study are to inventory fungi in farmers saved-seed samples of sorghum and pearl millet collected from various localities of Burkina Faso, to evaluate the effect of seed-borne inoculum of the main fungi recorded on seedlings emergence, seedlings mortality and diseases transmission and to investigate extracts from plants widely distributed in Swahilian and sudanian agro ecological zones, for their potential in improving seed quality and planting value of sorghum and pearl millet.

MATERIALS AND METHODS

Seed samples: A total of 86 seed samples of sorghum and 102 seed samples of pearl millet, weighted 500 to 1000 g each, were collected in 43 locations situated in the north-sudanian, south-sudanian and Swahilian zones of Burkina Faso (Fig. 1). Among the samples, 29 and 13 seed samples

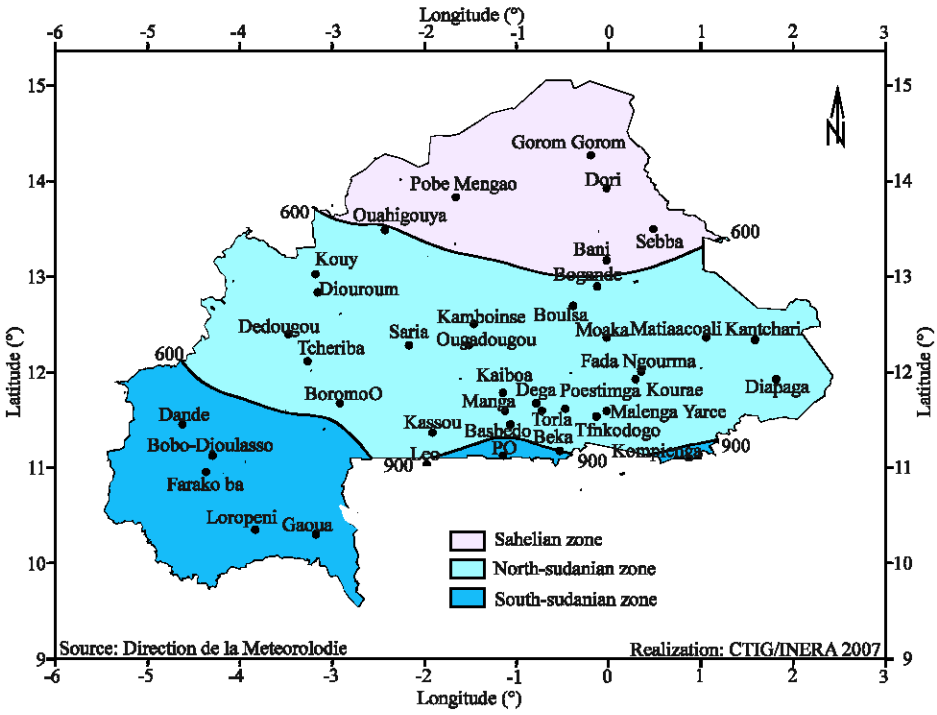

Fig. 1: Sites of collection of seed samples of sorghum and pearl millet in 1989 and 2002

of sorghum and pearl millet, respectively, were collected and tested in 1989 and data on eight major fungi recorded are analysed in the present study. Fifty seven and eighty nine seed samples of sorghum and pearl millet, respectively, were collected in 2002 and analysed in 2002 and 2003. The seed samples were packed in cotton or plastic bags and stored in a freezer at the Laboratory of Phytopathology of Kamboinsé research station in Burkina Faso, or, in a storage room at 5°C at the Danish Seed Health Centre for Developing Countries (DSHC) in Denmark, until use. Seventy six and 112 seed samples were tested, respectively at DSHC and at Kamboinsé research station.

Essential oils: Aerial parts of *Cymbopogon schoenanthus* (L.) Spreng., *Hyptis spicigera* Lam., *Lantana camara* L. and *Ocimum americanum* L., collected at Kamboinsé (in the Centre of Burkina Faso) and *C. nardus* Chiov. and *C. giganteus* Chiov., collected, respectively at Porto-Novo (in the north of Benin) and Koupéla (in the East of Burkina Faso), were air-dried for four days before oil isolation. The essential oils were extracted from the plant materials by the hydro distillation method using a Clevenger-type apparatus (Lamaty *et al*., 1987). The oils were separated from the water by decantation, dried over anhydrous sulphate and stored in darkness at 4°C.

Seed health testing methods: Three conventional seed health testing methods, reported by Mathur and Kongsdal (2003), were used to detect fungi associated with seeds:

Dry inspection method was used in this study to detect sclerotia of ergot in the seed samples. Fifteen and 90 g of seeds from each sample of pearl millet and sorghum, respectively, were visually inspected using a magnifying lamp. The sclerotia were selected from seeds, weighed and the result was expressed in percentage.

The standard blotter method was used to detect a wide range of fungi which are able to arise easily from seeds in presence of humidity. Four hundred untreated pure seeds from each sample were plated on moisten blotters in plastic Petri dishes at the rate of 25 seeds per dish and incubated for 7 days at 20-25°C under alternating cycles of 12 h near Ultraviolet Light (NUV) and 12 h darkness. Individual seeds were examined for the presence or absence of fungi under a stereomicroscope and identification was confirmed by examining mycelium and/or conidia under a compound microscope. The fungal species present on each seed were recorded and the percentage incidence of each fungus per sample was computed.

The washing test was used to detect teliospores of *S. cruenta*, *S. reiliana*, *S. sorghi* and *T. ehrenbergii* (causal agents of sorghum smuts), *T. penicillariae* (pearl millet smut) and oospores of *Peronosclerospora sorghi* and *Sclerospora graminicola* (causal agents of sorghum and pearl millet downy mildews), present on the surface of seeds. Sorghum seeds (5 g) and pearl millet seeds (10 g) were shaken in 25 mL of distilled water. The suspension was centrifuged at 3000 rpm for 5 min. The sediment was suspended in 1 mL of distilled water and examined under compound microscope. Teliospores of smuts and oospores of downy mildews were counted using a haemocytometer (type Fuchs-Rosenthal) and the total number of spores was calculated using the following formula (Mathur and Kongsdal, 2003):

$$\text{No. of spores in the seed sample} = \frac{\text{No. of spores} \times \text{vol. of spores suspension (mL)}}{\text{Counting area (mm}^2\text{)} \times \text{depth of counting area (mm)}}$$

with : 1 mm 3 = (1/1000) mL, so the formula can be written as follow:

$$\text{No. of spores in the seed sample} = \frac{\text{No. of spores} \times \text{vol. of spores suspension (mL)}}{\text{Counting area} \times \text{depth of counting area/1000 (mL)}}$$

Testing effect of major fungi on seedling emergence and mortality and evaluation of seed-to-seedling transmission: The effect of 17 and 15 fungi isolated, respectively from incubated seeds of sorghum and pearl millet was tested on seedling emergence and seedling mortality. One hundred and fifty seeds of sorghum and pearl millet previously disinfected in 70% ethanol for 5 min were inoculated by rolling on sporulating cultures of the different fungi, before sowing in pots (25 seeds per pot) containing sterilized soil, following the procedure used by Mathur *et al*. (1973). The pots were kept in a growing room for 10 days under 12 h fluorescent light/12 h darkness at 25-29°C. Untreated seeds, seeds disinfected with ethanol and seeds treated with a chemical called calthio (20% of Lindane, 25% of Thirame) were used as controls. For each crop, a randomized complete block design with three replicates of 50 seeds was used. Ten days after sowing, seedlings emergence and seedlings mortality were evaluated and percentage of emerged seedlings and percentage of dead seedling were calculated. To evaluate seed-to-seedling transmission of the fungi, ten seedlings from each treatment were cut at the level of the coleoptiles, disinfected in 70% ethanol for 2 min and plated on moistened blotter papers in plastic box for 5 days. Infected plants by the target fungus were counted using a stereomicroscope and the results

expressed in percentage. The severity of infection (the capacity of the fungus to propagate inside the seedling) was estimated on the incubated seedlings by assigning score based on the presence or absence of the fungus in plant parts: score 1 is attributed to healthy plants; score 2 to slightly infected plants (fungus present on plant stem); score 3 to highly infected plants (fungus present on plant stem and/or leaves). An index of severity was calculated following the formula used by Williams and Singh (1981) and the result was expressed in percentage:

$$S\,(\%) = \{\Sigma(xi - 1) / [E(xi) - 1] \times N\} \times 100$$

Where:

xi = Note attributed to each plant from the class i
ni = Number of plants from the class i
E(xi) = Range of the scale of notation (3)
N = Total number of observed seedlings (10)
S = Severity of infection or capacity of the fungus to invade the plant (%). Percentages of severity of infection were transformed into Arcsine values before performing the statistical analysis

Effect of essential oils on the radial growth *in vitro* of eight seed-borne fungi: Different PDA growth media containing 0.2% of essential oils (v/v) were prepared for growing the tested fungi. The oils were filtered with Millipore filters (Ø = 0.45 μm) for sterilization and mixed to autoclaved PDA and distributed in Petri dishes. Non treated PDA and PDA mixed with calthio at the rate of 0.25 g for 100 mL PDA, were considered as controls.

The pathogenic fungi selected by the previous tests were grown on PDA. For each fungus, a fungal disc measuring 4 mm diameter from a 5-day-old culture, was placed in a Petri dish containing the tested growth medium. Five replicates were used for each kind of growth medium. The Petri dishes were incubated under laboratory conditions (25-30°C) and two diameters perpendicular to each other of the colony were measured 4 and 6 days after incubation. The results were expressed in percent of mycelial inhibition calculated following the formula used by Singh *et al.* (2003):

$$\frac{dc - dt}{dc} \times 100$$

Where, dc and dt are average diameters of the mycelial colonies in the control and treated media, respectively.

Effect of essential oils on sorghum and pearl millet seed contamination by five fungi: The essential oils that showed potent growth inhibitory activity *in vitro* were tested for efficacy against *Colletotrichum graminicola*, *Curvularia* sp., *Exserohilum* sp., *Fusarium* sp. and *Phoma* sp., in naturally infected seeds of sorghum and pearl millet. Four doses: 5, 7.5, 10 and 15 μL of essential oil per gram of seeds were used. Before applying the oils, the seeds were mixed with 200 μL of distilled water in order to soften their teguments and allow essential oils absorption. The seeds were maintained hermetically closed in flasks for about 20 h (overnight) and evaluated for health using the standard blotter method described above. Untreated seeds, seeds mixed with distilled water and seeds treated with calthio at the rate of 2.5 g kg^{-1} of seeds, were used as controls. One hundred and fifty seeds were used for each treatment.

Data analyses: The main seed-borne fungi of sorghum and pearl millet, the effect of fungi on seedling emergence and seedling mortality, the main pathogenic and seed-transmitted fungi, the effect of essential oils on fungal growth and on seed infection were determined by Analyses of Variance (ANOVA) using a completely randomised design. The significance ($p<0.05$) of differences between treatments were determined, using the Duncan's Multiple Range (DMR) test of Statistical Analysis System, version 8.

RESULTS

Sorghum seed mycoflora: A total of thirty four fungal species belonging to seventeen genera (*Alternaria* Nees, *Bipolaris* Shoem. (syn. *Drechslera* fide Ellis), *Botryodiplodia* (Sacc.) Sacc., *Cercospora* Fres., *Colletotrichum* Corda, *Curvularia* Boedijn, *Drechslera* Ito, *Exserohilum* Leonard and Suggs., *Fusarium* Link, *Gloeocercospora* Bain and Edgerton ex Deighton, *Macrophomina* Petrak, *Myrothecium* Tode, *Nigrospora* Zimm., *Peronosclerospora* (Ito) Shirai and K. Hara, *Phoma* Sacc., *Sphacelotheca* de Bary, *Tolyposporium* Woronin ex Schröter) were detected in sorghum seed samples (Table 1). All the seed samples tested in 1989 and 2002/2003 were infected by *Phoma* sp and *F. moniliforme*, respectively. *F. moniliforme*, *C. lunata*, *C. graminicola*, *F. pallidoroseum* and *Drechslera* sp. were found in 59 to 93% of the samples in 1989, while *Phoma* sp. and *Curvularia lunata* were detected in 96.5 and 93% of the samples, respectively, in 2002/2003. *Sphacelotheca sorghi* and *Colletotrichum graminicola*, causing important diseases on sorghum, were encountered in, respectively 75.4 and 24.6% of the samples tested. Fifteen fungi occurred in few number of samples (1.7-5.3% of the samples).

All the seed samples tested using dry inspection were free of sclerotia of *Claviceps sorghi*, the causal agent of sorghum ergot.

Table 1: Fungal species detected in 86 seed samples of sorghum from Burkina Faso in 1989 and 2002/2003, by dry inspection, washing of seeds and blotter tests methods

Fungal species detected in seed samples of pearl sorghum	Percentage of infected samples		Range of infection (%) recorded in 2002/2003
	1989 (29)[a]	2002/2003 (89)[a]	
Alternata alternata (Fr.) Keissler	-	10.50	0.2-6.5
Alternata longissima Deighton and MacGarvie	-	15.80	0.2-5.0
Alternata raphani Groves and Skolko	-	5.30	-
Alternata sesami (Kawamaura) Mohanty and Bohera	-	3.50	-
Bipolaris maydis (Nisikado and Miyake) Shoem.	-	7.00	-
Bipolaris oryzae (Breda de Haan) Shoem.	-	3.50	-
Bipolaris sorghicola (Lefevre and Sherwin) Alcorn	-	1.70	-
Bipolaris sorokiniana (Sacc.) Shoem.	-	3.50	-
Bipolaris spicifera (Bainier) Subram.	-	5.30	-
Botryodiplodia theobrome Pat.	-	1.70	-
Cercospora sesami Zimm.	-	3.50	-
Colletotrichum graminicola (Ces.) Wilson	58.6	24.60	0.2-5.0
Curvularia cymbopogonis (C. W. Dodge) Groves and Skolko	-	5.30	-
Curvularia eragrostidis (P. Henn.) J. A. Meyer	-	24.60	0.2-7.0
Curvularia lunata (Wakk.) Boedijn	62.1	93.00	0.5-51.5
Curvularia oryzae Bugnicourt	-	5.30	-
Curvularia pallescens Boedijn	-	56.10	0.2-16.5
Curvularia trifolii (Kauffin.) Boedijn	-	7.00	-
Drechslera sp.	65.5	-	-
Exserohilum rostratum (Drechsler) Leonard and Suggs.	-	36.80	0.2-8.5
Fusarium equiseti (Corda) Sacc.	41.4	15.80	0.5-5.5
Fusarium moniliforme Sheldon	93.1	100.00	1.5-83.5
Fusarium pallidoroseum (Cooke) Sacc.	58.6	22.80	0.5-8.5
Fusarium solani (Mart.) Appel and Wollenw. emend. Snyder and Hansen	-	24.60	0.2-12.0
Fusarium subglutinans (Wollenw. and Reinking) Nelson, Taussoun and Marasas	-	10.50	0.2-3.5
Gloeocercospora sorghi Bain and Edgerton ex Deighton	37.9	8.80	0.5-1.7
Macrophomina phaseolina (Tassi) Goid.	-	5.30	-
Myrothecium roridum Tode ex Fr.	-	1.70	-
Nigrospora oryzae Zimm. (Berk. and Br.) Petch	-	5.30	-
Peronosclerospora sorghi (W. Weston and Uppal) C.G. Shaw	-	5.30	-
Phoma sp.	100.0	96.50	0.2-75
Sphacelotheca reiliana (Kühn) Clint.	-	5.30	-
Sphacelotheca sorghi (Link) Clint.	-	75.40	63.0-105469[b]
Tolyposporium ehrenbergii (Kühn) Patouillard	-	33.30	-

[a]: No. in the parentheses represent the number of seed samples tested, [b]: No. of spores per gram of seed

In the three ecological zones, *Phoma* sp., *F. moniliforme*, *C. lunata* and *S. sorghi* encountered in 72 to 100% of the samples were the widest spread fungi (Table 3). All of them, excepted *S. sorghi*, occurred more frequently in the north and south-sudanian zones than in the Swahilian zone. Among the major fungal species recorded in sorghum seeds five were not encountered in the Swahilian zone. In addition, seed samples from the Swahilian zone had the lowest rates of infection compared to those from the north and south-sudanian zones.

Pearl millet seed mycoflora: Twenty seven fungal species were associated with seed samples of pearl millet (Table 2). In 1989, *F. moniliforme*, *Phoma* sp. and *Drechslera* sp. were associated to all the samples, at high levels of infection. *F. equiseti* and *F. pallidoroseum* were also encountered frequently. Among the fungi recorded in 2002/2003, *Phoma* sp., *F. moniliforme*, *T. penicillariae* and *C. lunata* were encountered, respectively in 100, 95.4, 88.2 and 82.9% of the samples (Table 2). Other fungi with high frequency of dispersal included *C. pallescens*, *E. rostratum*, *S. graminicola* and *C. fusiformis* (Table 2).

T. penicillariae, *F. moniliforme* and *A. strictum* occurred at high levels in seeds from Swahilian zone. High infection levels of *Phoma* sp. ranging from 33.70-34.85% infection were recorded on seed samples from the three agro ecological zones (Table 4). The widest spread fungi in the three zones were *Phoma* sp., *F. moniliforme*, *T. penicillariae* and *C. lunata* with 66 to 100% of seed samples infected.

During examination of incubated seeds of sorghum and pearl millet, a number of saprophytic fungi, not reported in the tables, were present in the seed samples. These included mainly *Aspergillus niger* Tiegh., *A. flavus* Link, *Cladosporium* sp., *Penicillium* sp. and *Rhizopus* sp.

Table 2: Fungal species detected in 102 seed samples of pearl millet from Burkina Faso in 1989 and 2002/2003, by dry inspection, washing of seeds and blotter tests methods

Fungal species detected in seed samples of pearl millet	Percentage of infected samples		Range of infection (%) recorded in 2002/2003
	1989 (13)[a]	2002/2003 (89)[a]	
Acremonium strictum (Corda) W. Gams	-	12.3	0.5-6.0
Alternata alternata (Fr.) Keissler	-	2.2	-
Alternata longissima Deighton and MacGarvie	-	2.2	-
Alternata raphani Groves and Skolko	-	5.6	-
Alternata sesami (Kawamaura) Mohanty and Bohera	-	4.5	-
Bipolaris maydis (Nisikado and Miyake) Shoem.	-	12.3	0.3-3.0
Bipolaris oryzae (Breda de Haan) Shoem.	-	6.7	-
Bipolaris sorokiniana (Sacc.) Shoem.	-	1.1	-
Claviceps fusiformis Loveless	-	31.9	0.001-0.4[b]
Colletotrichum sp.	-	2.2	-
Curvularia cymbopogonis (C.W. Dodge) Groves and Skolko	-	4.5	-
Curvularia eragrostidis (P. Henn.) J. A. Meyer	-	14.6	0.3-5.5
Curvularia lunata (Wakk.) Boedijn	46.1	82.9	0.5-34.0
Curvularia oryzae Bugnicourt	-	2.2	-
Curvularia pallescens Boedijn	-	65.9	0.3-10.5
Drechslera sp.	100.0	-	-
Exserohilum rostratum (Drechsler) Leonard and Suggs.	-	55.7	0.3 -16.0
Fusarium eqniseti (Corda) Sacc.	53.8	21.6	0.5-5.0
Fusarium moniliforme Sheldon	100.0	95.4	0.5-62.0
Fusarium pallidoroseum (Cooke) Sacc.	76.9	9.1	0.5-22.5
Fusarium solani (Mart.) Appel and Wollenw. emend. Snyder and Hansen	-	3.4	0.3-9.0
Fusarium subglutinans (Wollenw. and Reinking) Nelson, Taussoun and Marasas	-	17.0	0.3-13.0
Melanospora zimiae Corda	-	1.1	-
Phoma sp.	100.0	100.0	2.0-93.0
Rhizoctonia solani Kühn	-	2.2	-
Sclerospora graminicola (Sacc.) Schoet.	-	41.4	5.0-125.00[c]
Tolyposporium penicillariae Bref.	-	88.2	187.5-13563[c]

[a]: No. in the parentheses represent the number of seed samples tested, [b]: Percentage of sclerotia by weight of seed, [c]: No. of spores per gram of seed

Table 3: Major seed-borne fungi recorded in 86 seed samples of sorghum grown in three agro ecological zones in Burkina Faso in 1989 and 2002

	Infection (%)														*S. sorghi* (Teliospores
Agro ecological zones	Pspp.	Fm	Cl	Cg	Cp	Fs	Gloe	Er	Fpalli	Fe	Ce	Aalt	Along	Fsub	g^{-1} of seed)
North sudanian zone	31.97b	19.30a	10.19a	2.61a	2.61a	0.88a	0.46a	0.74a	1.32a	0.49a	0.63a	0.29a	0.37a	0.32a	3.681b
[64]	(96.87)	(96.87)	(84.37)	(31.25)	(55.31)	(23.40)	(17.18)	(29.78)	(35.93)	(21.87)	(21.27)	(10.63)	(19.14)	(12.76)	(72.34)
South-sudanian zone	41.14a	26.50a	9.80a	2.50a	2.70a	1.05a	0.19a	2.40a	2.13a	0.55a	0.30a	0.15a	0.00a	0.00a	1625b
[17]	(100.00)	(100.00)	(82.35)	(64.70)	(100.00)	(60.00)	(29.41)	(40.00)	(47.05)	(41.17)	(60.00)	(20.00)	(0.00)	(0.00)	(100.0)
Sahelian zone	14.15c	5.20b	4.05b	0.00a	0.65b	0.00a	0.00a	2.40a	0.00b	0.10a	0.25a	0.00a	0.00a	0.00a	16274a
[5]	(100.00)	(100.00)	(80.00)	(0.00)	(80.00)	(0.00)	(0.00)	(80.00)	(0.00)	(20.00)	(20.00)	(0.00)	(0.00)	(0.00)	(80.0)
Average	32.75	19.90	9.76	2.43	2.44	0.80	0.38	1.03	1.40	0.48	0.57	0.25	0.30	0.26	4605.59
Standarderror	20.69	17.64	10.30	7.91	3.73	2.21	1.41	2.20	2.98	1.31	1.65	1.12	1.12	0.94	16316.56

Psp. : *Phoma* sp.; Fm: *Fusarium moniliforme*; Cl: *Curvularia lunata*; Cg: *Colletotrichum graminicola*; Cp: *Curvularia pallescens*; Fs: *Fusarium solani*; Gloe: *Gloeocercospora sorghi*; Er: *Exserohilum rostratum*; Fpalli: *Fusarium pallidoroseum*; Fe: *Fusarium equiseti*; Ce: *Curvularia eragrostidis*; Aalt: *Alternaria alternata*; Along: *Alternaria longissima*; Fsub: *Fusarium subglutinans*; Ssorg: *Sphacelotheca sorghi*, Means followed by the same letter within the same column are not significantly different at level 5%, ( ): No. of infected seed samples (%), [ ]: No. of tested seed samples

Effect of seed-borne inoculum on seedling emergence, seedling mortality and disease transmission: Results of the infection experiments performed on sorghum fungi are shown in Table 5. All fungi tested had no significant effect on emergence of sorghum seedlings. *C. oryzae* and *F. moniliforme* caused the highest mortality rates in seedlings (54-65.3%) of sorghum, followed by *F. equiseti* (34%). Examination of incubated seedlings revealed that *F. moniliforme*, *F. equiseti*, *C. oryzae* and *F. subglutinans* had the highest levels of seed-transmittance with 96.7 to 100% transmission rates. Other fungi with high frequency of transmission included *F. solani*, *C. trifolii*, *C. pallescens*, *E. rostratum* and *C. lunata* (Table 5). The evaluation of severity of infection on sorghum seedlings revealed that *C. oryzae* (46.7% of severity) was the most pathogenic fungus and *N. oryzae* (0% of severity) was the least pathogenic fungus.

On pearl millet, only *F. solani* caused a negative influence on seedlings emergence compared to the chemical control (Table 6). High numbers of dead plants were observed with *F. solani* (19.3%), *A. strictum* (18.7%)

Table 4: Major seed-borne fungi recorded in 101seed samples of pearl millet grown in three agro ecological zones in Burkina Faso in 1989 and 2002

	Infection (%)												Sgram (oospores per g of seed)	Cfusi (% of sclerotia)	Tpenic (Teliospores g^{-1} of seed)
Agro ecological zones	Psp.	Fm	Cl	Bm	Cp	Fs	As	Er	Fpalli	Fe	Ce	Fsub			
North-sudanianzone	33.99a	13.97b	6.45a	0.16b	1.11a	0.19a	0.25b	2.75a	1.38a	0.49a	0.48a	0.89a	17.70a	0.09a	1509.90b
[80]	(100.00)	(100.00)	(82.27)	(5.97)	(29.85)	(2.98)	(11.94)	(55.22)	(20.25)	(22.78)	(17.91)	(22.38)	(40.98)	(36.53)	(88.23)
South-sudanianzone	34.85a	12.20b	6.85a	0.41a	0.30b	0.08a	0.16b	1.41a	0.22a	0.70a	0.05a	0.00a	5.93a	0.01a	1307.10b
[10]	(100.00)	(90.00)	(90.00)	(33.33)	(22.22)	(11.11)	(11.11)	(55.55)	(20.00)	(20.00)	(11.11)	(0.00)	(50.00)	(20.00)	100.00)
Sahelianzone	33.70a	21.00a	5.20a	0.04b	0.00b	0.06a	0.75a	2.25a	0.12a	0.33a	0.00a	0.02a	16.25a	0.01a	2821.30a
[12]	(100.00)	(100.00)	(66.00)	(8.33)	(0.00)	(8.33)	(25.00)	(50.00)	(8.33)	(25.00)	(0.00)	(8.33)	(50.00)	(8.33)	(80.00)
Average	34.04	14.63	6.34	0.17	0.88	0.16	0.31	2.54	1.12	0.49	0.37	0.68	16.32	0.07	1739.21
Standarderror	22.25	13.01	7.38	0.68	2.15	1.22	1.18	3.93	3.83	1.31	1.25	2.34	36.20	0.41	2361.45

Psp. : *Phoma* sp.; Fm: *Fusarium moniliforme*; Cl: *Curvularia lunata*; Bm: *Bipolaris maydis*; Cp: *Curvularia pallescens*; Fs: *Fusarium solani*; As: *Acremonium strictum*; Er: *Exserohilum rostratum*; Fpalli: *Fusarium pallidoroseum*; Fe: *Fusarium equiseti*; Ce: *Curvularia eragrostidis*; Fsub: *Fusarium subglutinans*; Sgram: *Sclerospora graminicola*; Cfusi: *Claviceps fusiformis*; Tpenic: *Tolyposporium penicillariae*, *: Means followed by the same letter within the same column are not significantly different at level 5%, (): No. of infected seed samples (%), [ ]: No. of tested seed samples

Table 5: Seedlings emergence, seedlings mortality, disease transmission and severity of infection recorded after sowing inoculated seed of sorghum by different fungal species

Treatments	Seedlings emergence rate (%)	Percentage of dead seedlings 10 days after inoculation	Percentage of infected seedlings 10 days after inoculation	Index of severity (%)
Bipolaris oryzae	90.0a	3.3c	33.3bcd	17.8abcd (0.2)
Bipolaris maydis	84.7a	0.7c	10.0cd	3.3cd (0.1)
Bipolaris sorokiniana	88.7a	0.0c	43.3abcd	16.7abcd (0.2)
Bipolaris spicifera	95.3a	1.3c	36.7bcd	16.7abcd (0.2)
Colletotrichum graminicola	96.0a	3.3c	6.7cd	2.2cd (0.2)
Curvularia lunata	94.7a	6.0c	62.7abc	24.9abcd (0.2)
Curvularia eragrostidis	90.0a	6.0c	36.7bcd	13.3bcd (0.1)
Curvularia oryzae	83.3a	65.3a	96.7a	46.7a (0.5)
Curvularia pallescens	94.0a	0.0c	75.2ab	31.3abc (0.3)
Curvularia trifolii	88.0a	0.7c	76.7ab	32.2abc (0.3)
Exserohilum rostratum	89.3a	9.3c	63.3abc	28.9abcd (0.3)
Fuasrium moniliforme	84.7a	54.0a	100.0a	33.5abc (0.3)
Fusarium solani	91.0a	4.7c	86.7ab	33.3abc (0.3)
Fusarium subglutinans	88.0a	15.3c	96.7a	43.3ab (0.4)
Fusarium equiseti	90.7a	34.0b	100.0a	44.4ab (0.5)
Nigrospora oryzae	95.3a	0.7c	0.0d	0.0d (0.0)
Phoma sorghina	92.0a	0.7c	33.3bcd	14.4bcd (0.1)
Untreated seed (control)	95.3a	2.0c	33.3bcd	15.5bcd (0.1)
Disinfected seed (ethanol)	91.3a	3.3c	63.3abc	35.6ab (0.4)
Chemical control	92.7a	0.0c	46.7abcd	17.8abcd (0.2)
Mean	90.5	13.2	55.1	23.6 (0.2)
CV(%)	4.9	69.9	32.4	45.5

Means followed by the same letter within the same column are not significantly different at level 5%, No. in the parentheses represent the percentages of severity of infection turned into Arcsines values on which statistical analysis was performed

and *F. moniliforme* (18%). Results on disease transmission revealed that *F. solani* was the most seed-transmitted fungus (100% transmission rate). Other fungi with high levels of seed-transmittance included *F. moniliforme*, *A. strictum*, *C. eragrostidis* and *C. lunata*. The highest index of severity (50%) was recorded with *F. solani* while the lowest was obtained with *P. sorghina* (4.4%). A. *strictum* (44.4%) and *F. moniliforme* (43.3%) infected severely the seedlings.

Inhibitory effect of essential oils on the mycelial growth of eight major seed-borne fungi: Essential oils of *C. schoenanthus*, *C. nardus* and *C. giganteus* reduced the mycelial growth of all the fungi at the rates of 86-100% and 85-100%, respectively, at 4 and 6 days after incubation (DAI) (Table 7). The percent mycelial inhibition obtained with these oils on *F. solani*, *C. lunata*, *E. rostratum* and *P. sorghina* were similar to those obtained with the chemical control. In addition these essential oils reduced better the mycelial growth of *F. moniliforme*, *C. graminicola*, *B. sorokiniana* and *A. strictum* than the chemical control. Essential oil of *C. nardus* completely inhibited the mycelial growth of all the fungi tested both at 4 and 6 DAI. Percent of growth inhibition due to *L. camara*, *O. americanum* and *H. spicigera* were relatively low (less than 50%). The growth of *P. sorghina*, *F. solani*, *A. strictum* and *B. sorokiniana* was favoured by essential of *L. camara* (negative values of percent of mycelial inhibition).

Table 6: Seedlings emergence, seedlings mortality, disease transmission and severity of infection recorded after sowing inoculated seed of pearl millet by different fungal species

Treatments	Seedlings emergence rate (%)	Percentage of dead seedlings 10 days after inoculation	Percentage of infected seedlings 10 days after inoculation	Index of severity (%)
Acremonium strictum	90.0ab	18.7a	93.3ab	44.4ab (0.5)
Bipolaris maydis	94.0ab	0.0b	23.3f	11.1ef (0.1)
Bipolaris sorokiniana	92.7ab	0.7b	19.0f	6.4f (0.1)
Curvularia eragrostidis	96.7ab	1.3b	86.7ab	38.9abc (0.4)
Curvularia lunata	88.0ab	3.3b	80.0ab	34.4bcd (0.3)
Curvularia pallesceus	96.0ab	1.3b	56.7bcde	22.2def (0.2)
Colletotrichum sp.	93.3ab	1.3b	26.7ef	8.9f (0.1)
Exserohilum rostratum	90.0ab	1.3b	56.7bcde	21.1def (0.2)
Fuasrium moniliforme	87.3ab	18.0a	93.3ab	43.3ab (0.4)
Fusarium solani	84.7b	19.3a	100.0a	50.0a (0.5)
Fusarium pallidoroseum	86.7ab	0.0b	60.0bcd	21.1def (0.2)
Fusarium subglutinans	91.3ab	0.7b	40.0cdef	14.4ef (0.1)
Myrethecium roridum	94.7ab	0.7b	30.0def	10.0f (0.1)
Phoma sorghina	90.0ab	0.7b	13.3f	4.4f (0.1)
Rhizoctonia solani	92.7ab	2.0b	20.0f	6.7f (0.1)
Untreated seed (control)	91.3ab	0.0b	83.3ab	33.3bcd (0.3)
Disinfected seed (ethanol)	92.7ab	0.0b	90.0ab	35.5bcd (0.4)
Chemical control	97.3a	0.7b	63.3bc	26.7cde (0.3)
Mean	91.6	3.9	57.5	24.0 (0.2)
CV (%)	4.7	77.7	21.8	26.8

Means followed by the same letter within the same column are not significantly different at level 5%, No. in the parentheses represent the percentages of severity of infection turned into Arcsines values on which statistical analysis was performed

Table 7: Inhibitory effect of different essential oils on the radial growth *in vitro* of eight seed-borne fungi at 4 and 6 Days after Incubation (DAI)

	Percentage inhibition of radial growth of fungi							
	Fusarium moniliforme		*Curvularia lunata*		*Exserohilum rostratum*		*Fusarium solani*	
Treatments	4 DAI	6 DAI	4 DAI	6 DAI	4 DAI	6 DAI	4 dai	6 DAI
CC	76.56b	77.25b	94.88a	94.10a	100.00a	96.25a	77.30b	84.68b
Cg	98.26a	97.50a	98.15a	98.59a	100.00a	100.00a	88.89ab	86.20b
Cn	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
Cs	100.00a	94.87a	97.78a	96.90a	100.00a	100.00a	98.67a	95.27ab
Hs	11.76c	-7.00cd	37.52c	12.28c	13.48d	14.80c	4.14c	7.13c
Oa	-8.69e	-11.01d	25.44d	19.36c	22.83c	21.44c	-23.29d	8.37c
Lc	0.03d	-15.60d	57.52b	48.83b	35.02b	36.82b	-38.43d	-19.85d
C	0.00d	0.00c	0.00e	0.00d	0.00e	0.00d	0.00c	0.00c
Mean	47.24	42.00	63.91	58.76	58.91	58.66	38.40	45.22
Standard error	6.18	6.80	6.62	7.89	5.48	5.82	13.72	9.69
	Colletotrichum graminicola		*Bipolaris sorokiniana*		*Phoma sorghina*		*Acremonium strictum*	
Treatments	4 JAI	6 Jai	4 DAI	6 DAI	4 DAI	6 DAI	4 DJAI	6 DAI
CC	57.01b	67.21c	72.63b	73.32b	91.66a	89.99a	86.02b	88.54b
Cg	85.68a	84.88b	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
Cn	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
Cs	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
Hs	23.76d	17.49f	-20.93d	-49.57d	39.09b	8.33b	22.84c	27.40cd
Oa	26.76d	38.25d	8.09c	-1.40c	12.12c	8.20b	13.11d	33.44c
Lc	41.42c	47.60d	-37.03e	-51.57d	-111.66d	-188.33c	7.72de	24.50d
C	0.00e	0.00g	0.00c	0.00c	0.00c	0.00b	0.00e	0.00e
Mean	54.33	56.93	40.34	33.84	41.40	27.27	53.71	60.05
Standard error	11.25	5.59	8.56	7.49	16.74	13.32	7.40	6.20

CC = Chemical Control; Cg = *Cymbopogon giganteus*; Cn = *Cymbopogon nardus*; Cs = *Cymbopogon schoenanthus*; Hs = *Hyptis spicigera*; Oa = *Ocimum americanum*; Lc = *Lantana camara*; C = Control (untreated seeds), Means followed by the same letter within the same column are not significantly different at level 5%

Efficacy of essential oils in controlling seed-borne fungi of sorghum and pearl millet: A seed health testing was performed after the treatment of the seeds by essential oils of *C. nardus*, *C. giganteus* and *C. schoenanthus* and percent of infected seeds by *Phoma* sp., *Fusarium* sp., *Curvularia* sp., *Colletotrichum graminicola* and *Exserohilum* sp. were recorded. The results presented in Table 8 showed that all the essential oils reduced significantly sorghum and pearl millet seed contamination by the selected fungi. The lowest rates of infected seeds

Table 8: Effect of treatment by essential oils of *C. giganteus*, *C. schoenanthus* and *C. nardus* at different doses on the percent of infected seeds by five fungi involved in sorghum and pearl millet seedlings infections in Burkina Faso

	Percent of infected seeds														
	Controls			*C. giganteus*				*C. schoenanthus*				*C. nardus*			
Pathogens	C	WC	CC	D1	D2	D3	D4	D1	D2	D3	D4	D1	D2	D3	D4
Sorghum															
Phoma sp.	66.7	73.3	17.3	14.0	13.3	0.7	2.0	25.3	18.0	10.0	3.3	12.0	8.0	5.3	3.3
Fusarium sp.	19.3	9.3	2.7	7.3	14.7	4.0	4.0	17.3	15.3	3.3	4.7	12.7	10.0	6.0	2.7
Curvularia sp.	12.7	36.0	8.7	16.7	11.3	1.3	0.0	20.7	21.3	6.7	6.5	6.7	9.3	4.7	1.3
C. graminicola	23.3	19.3	13.4	26.7	11.3	10.7	12.0	10.0	11.3	11.3	9.3	16.0	14.7	4.0	4.7
Pearl millet															
Phoma sp.	66.7	61.1	9.3	11.3	6.0	0.7	0.0	44.0	30.7	22.0	3.3	20.7	14.7	6.0	3.3
Fusarium sp.	35.3	14.3	22.7	14.0	3.3	5.3	0.0	9.3	8.0	14.7	12.0	7.3	10.7	8.0	2.7
Curvularia sp.	40.0	35.8	34.0	21.3	17.3	0.7	0.0	32.0	50.0	24.7	15.3	24.7	23.3	6.0	0.7
Exserohilum sp.	7.3	9.1	4.0	4.0	6.7	0.0	0.0	4.7	5.3	5.3	2.7	10.0	3.3	2.0	0.7

C = Untreated seeds; WC = Water Control (seeds mixed with distilled water); CC = Chemical control (seeds treated with chemical); D1 = 5 μL of essential oil per g of seeds; D2 = 7.5 μL of essential oil per g of seeds; D3 = 10 μL of essential oil per g of seeds; D4 = 15 μL of essential oil per g of seeds

were generally recorded on seeds treated with 10 μL and/or 15 μL of essential per g of seeds. In most of the time, these doses were most efficient or so efficient as the chemical control. Oil of *C. giganteus* used at 15 μL g^{-1} of seeds eliminated completely pearl millet seed contamination by all the fungi tested. However, the dose 15 μL of essential oil g^{-1} strongly reduced seed germination in Petri dishes.

DISCUSSION

Good seed is recognized as an important input in any agricultural production system. One of the important aspects of good seed, besides high germination and purity, is the absence of seed-borne pathogens. At least 27 fungal species were encountered in high frequencies and infection percentages in different samples of sorghum and pearl millet collected from farmers own saved-seed in Burkina Faso (Table 1, 2). *Phoma* sp., *F. moniliforme*, *C. lunata*, *S. sorghi*, *T. penicillariae*, *E. rostratum*, *S. graminicola* and *C. graminicola* were the main fungi occurring frequently in sorghum and/or pearl millet seeds. Out of the fungi, *S. sorghi*, *S. reilana*, *T. ehrenbergii*, *T. penicillariae*, *S. graminicola*, important pathogens whose inoculum is present on the surface of seeds, were recorded for the first time in farmers saved-seeds of sorghum and pearl millet from Burkina Faso. About ten fungal species belonging to the genera *Fusarium*, *Phoma*, *Curvularia* and *Exserohilum* heavily infected both sorghum and pearl millet seeds. In previous surveys, Mathur *et al.* (1975) and Tarp *et al.* (1987) also found high frequencies of occurrence of *Phoma* sp., *F. moniliforme* and *C. lunata* in sorghum seeds from India and Mozambique. Beyond seed rots and seedling blights induced by these fungi, their presence at high levels in seeds is alarming since the genera *Fusarium* and *Phoma* are known to produce mycotoxins (CAST, 1989; Mathur and Manandhar, 2003).

Anthracnose and pearl millet downy mildew were frequently reported in the literature to be the most widespread diseases on sorghum and pearl millet in many parts of the world (ICRISAT, 1980; Singh, 1995) and in Burkina Faso (Kaboré and Couture, 1983; Séréme, 1995). The present survey indicated that sorghum covered smut and pearl millet smut caused by *S. sorghi* and *T. penicillariae*, respectively, were widely scattered since their seed-borne inoculum were encountered more frequently in the samples than those of anthracnose (*C. graminicola*) and downy mildew (*S. graminicola*). Even if the role of secondary inoculum in anthracnose and downy mildew spread in field is of great importance (Warren, 1986; Singh and Williams, 1980), field surveys will be needed to complete the seed health surveys and to follow the evolution of the important diseases caused by these fungi. Other studies will also be necessary to establish the relationship between the amount of seed-borne inoculum of important pathogens such as *S. sorghi*, *C. graminicola* and *S. graminicola* in sorghum and pearl millet and the severity of the diseases its can produce in fields.

Occurring of all the major fungi in both sorghum and pearl millet seeds from the north-sudanian zone could be related to the large number of seed samples coming from this agro ecological zone. In addition, the sites of collection of the samples situated in the north-sudanian zone were more various than in the two other zones. In an other hand, the climate, drier in the Swahilian zone than the north and south-sudanian zones could justify the low representation of fungal species in the Swahilian zone. That was illustrated by, for example, the occurrence of *C. graminicola*, *G. sorghi*, *F. solani* and *F. pallidoroseum* in sorghum seeds from the north and south-sudanian zones and their absence in the seeds from the Swahilian zone.

According to the infection experiments carried out with the main fungi recorded by the blotter test, none of

these fungi, except *F. solani* on pearl millet, had significant effect on sorghum and pearl millet seedlings emergence. These results were not in accordance with those previously reported by Mathur *et al.* (1973). These researchers observed reduction in germination rate of pearl millet by inoculating seeds with *C. lunata*, *E. rostratum*, *B. spicifera* and *F. equiseti*. Testing for germination of naturally infected seeds and seeds infected artificially at different dates could provide more information on the effect of these fungi on seedling establishment. Many fungi including *F. moniliforme*, *F. equiseti*, *F. subglutinans*, *C. oryzae*, A. *strictum*, *F. solani*, *C. pallescens*, *E. rostratum* and *C. lunata*, were frequently transmitted to sorghum and pearl millet seedlings through infected seeds. Among the seed-transmitted fungi, *F. moniliforme*, *F. equiseti*, *F. subglutinans*, *C. oryzae*, A. *strictum* and *F. solani*, caused high rates of dead seedlings. These results indicated that these seed-transmitted fungi could be the main fungal pathogens involved in sorghum and pearl millet seedlings diseases in Burkina Faso. In spite *Phoma* sp. being identified as the most frequent fungus recorded in the seeds, it did not appear to be so pathogenic (Table 6, 7). However, considering the high infection levels of *Phoma* sp. encountered in the seeds, further studies are necessary to elucidate the exact role of this fungus in seed. In contrast to *Phoma sorghina*, fungi as *F. equiseti*, *F. solani*, *C. oryzae* and *A. strictum*, occurring in low frequencies in seeds, were found to be very pathogenic to seedlings. Their presence in seeds, even in low levels, could be prejudicial to the seed lot. The main fungal pathogens involved in sorghum and pearl millet seedling diseases in Burkina Faso included the genera *Acremonium*, *Curvularia*, *Fusarium* and *Exserohilum*. Occurrence of these pathogens in many samples suggested that the pathogens were widely distributed in Burkina Faso.

Among the essential oils tested against the fungi, those extracted from *C. giganteus*, *C. nardus* and *C. schoenanthus* exhibited anti fungal activity by inhibiting *in vitro*, the mycelial growth of the tested fungi. In previous studies, Dawson-Andoh *et al.* (2000) observed an inhibitory effect of essential oil of *C. nardus* on *Alternaria alternata* and *Aureobastum pullulans*, two pathogenic fungal on wood. The Japan International Research Center for Agricultural Science (JIRCAS) (2005) also reported that oil of *C. nardus* completely inhibited the growth of food stocks fungi such as *Aspergillus candidus*, *A. flavus*, *A. versicolor*, *Eurotium amstelodami*, *E. chevalieri*, *Penicillium adametzii*, *P. citrinum*, *P. griseofulvum* and *P. islandicum*. By reducing *in vivo*, the seed contamination by the major fungi, the essential oils of *C. giganteus*, *C. nardus* and *C. schoenanthus*, confirmed their anti fungal properties and seemed to be the most potent extracts usable in seed-borne fungi management.

CONCLUSIONS

The presence of so many pathogenic fungi at high levels in farmers saved-seed from various geographical areas indicates a clear need for field surveys for these and other pathogens. There also is a clear need to increase public awareness on aspects related to seed health and to develop suitable management practices for improving the quality of the seeds. Testing seed health of major crops should be introduced in the national seed quality control system.

Plant species such as *C. giganteus*, *C. nardus* and *C. schoenanthus*, potent in controlling cereal crop seed-borne fungi, are available in all over tropical zone. The cost of intensive use of their essential oils should be relatively low and affordable to poor communities in many sorghum and pearl millet producing areas. Nevertheless, efficacy of these essential oils should be evaluated under field conditions. Moreover, the active constituents should be determined and quantified and the mammal toxicity tested.

ACKNOWLEDGMENTS

The authors are grateful to the Danish Seed Health Centre for Developing Countries (DSHC) for providing laboratory equipment, technical assistance and funds for research. The authors are very thankful to the International Foundation for Science for providing financial support and complementary equipment as a research grant given to Mrs Zoma-Zida P. Elisabeth. They wish to acknowledge Dr. Jan Torp (DSHC, Denmark) and Dr. Hamidou Traoré (INERA, Burkina Faso) for going through the manuscript and giving us their valuable comments and suggestions and Dr. Nébié Roger (IRSAT, Burkina Faso) for extracting essential oils.

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