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Antifungal Effect of *Cymbopogon citratus*, *Eucalyptus camaldulensis* and *Azadirachta indica* Oil Extracts on Sorghum Seed-Borne Fungi

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Abstract: The aim of the study was to evaluate the potential of essential of from local plant in controlling some major seed-borne fungi of sorghum grow in Burkina Faso. Essential oils from *Cymbopogon citratus* (Lemongrass) *Eucalyptus camaldulensis*, (Eucalyptus) and crude oil from *Azadirachta indica* (Neem) were tested *in vitro* for inhibitory activity against *Colletotrichum graminicola*, *Phoma sorghina* and *Fusarium moniliforme*. Plant extracts were also tested on naturally infected sorghum seeds for controlling the fungi above mentioned. Essential oil from *C. citratus* significantly inhibited the *in vitro* radial growth of *C. graminicola* (76.2% inhibition), compared to the fungicide Dithane M-45. The mycelial growth of *P. sorghina* and *F. moniliforme* was slightly affected by this oil at the concentrations used. The extent of inhibition of the fungal growth was dependent on the concentration of essential oil used. Neem crude oil and Eucalyptus essential oil presented low inhibitory activity against test fungi. Concentrations of Eucalyptus essential oil were not harmful to sorghum seedling growth, while neem crude oil was highly phytotoxic. Essential oil of lemongrass at the concentration of 6% was effective in controlling seed-borne infection and seed-to-seedling transmission of *C. graminicola* and *P. sorghina* without affecting seedling development. Lemongrass has the potential to be used as sorghum seed treatment for controlling *C. graminicola*, *P. sorghina* and *F. moniliforme*.

Key words: Essential oils, crude oil, seed-borne fungi, seed treatment, seed transmission, sorghum

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is widely grown in the Soudano-Sahelian and Sahelian zones of West Africa. It constitutes, together with pearl millet, the staple food of most populations in these areas (Gueye and Delobel, 1999). Based on 1984-1998 averages, the production of sorghum is estimated to 49% of cereals grown in Burkina Faso. Abiotic and biotic stresses limit sorghum production. Leaf anthracnose, grain anthracnose and grain mould are the most important constraints of sorghum production in Burkina Faso. It was reported that the combined effect of leaf and stalk infection by *Colletotrichum graminicola* (Ces.) Wilson can cause yield losses ranging from 8 to 46%. Mould fungi such as *Fusarium moniliforme* Sheldon (Syn. *F. thapsinum* Klittich, Leslie, Nelson et Marasas sp. nov. (Klittich *et al*., 1997)) and *Phoma sorghina* (Sacc.) Boerema, Dorebosch and van Kesteren were found at high incidence on seed samples from Burkina Faso tested at the Danish Seed Health Centre (former DGISP) (Somda, unpublished data).

Good quality seed of improved varieties does not reach the traditional small-scale farmers. They use their own seeds extracted from plants without paying attention to diseased or healthy plants. Traditionally, smallholder farmers do not apply chemical seed treatment for the control of sorghum diseases because they cannot afford it. Widespread public concern for health and environmental effects of synthetic pesticides and the restriction of their use create an opportunity for alternative products, in particular reduced-risk pesticides (Isman, 2000; Alvarez-Castellenos *et al*., 2001). Research on natural products including plant extracts which might substitute agrochemicals or contribute to the development of new agents (Addor, 1995) cited by Alvarez-Castellenos *et al*. (2001) for disease control is

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extremely important. Many authors have shown that certain essential oils have properties against some important plant pathogens (Montes-Belmont and Carvajal, 1998; Alvarez-Castellenos *et al*., 2001; Soliman and Badea, 2002; Daferera *et al*., 2003; Nguefack *et al*., 2004; Nguefack *et al*., 2005). In developing countries, the use of plant parts or derivatives such as wood-ash to control insect pests of stored products and backyard vegetables has been practiced (Owusu, 2001). In Nigeria, lemongrass powder and essential oil have effectively protected melon seeds against toxigenic *Aspergillus flavus* (Banjole and Joda, 2004). Neem seed crude oil and leaf extracts efficiently controlled rice blast *in vitro* and *in vivo* (Amadioha, 2000). Neem crude oil and other plant derivatives have been used by smallholder farmers to protect their own-saved seed, grain and legumes against insect pests in Burkina Faso (Sérémé Paco, personal communication). Although many workers in developing countries reported on antifungal properties of plant extracts, potential of local plants needs to be documented in Burkina Faso. Therefore, the aim of this investigation, is to test the effectiveness of oil extracts of botanicals namely *Cymbopogon citratus* (D.C.) Stapf., *Eucalyptus camaldulensis* Dehnh. and *Azadirachta indica* A. Juss. in controlling sorghum seed-borne infection by *C. graminicola*, *F. moniliforme* and *P. sorghina* and investigate the effects of extracts on seedling growth and seed-to-seedling transmission.

MATERIALS AND METHODS

Fungal isolates: Isolates of *Colletotrichum graminicola*, *Fusarium moniliforme* and *Phoma sorghina* were recovered, in 2003 at DSHC (former DGISP), Denmark, from sorghum seeds collected in 2002 in Burkina Faso. The isolates were preserved on dry sterile soil and sand mixture in glass tubes for long term storage. A dense suspension (2-5 mL) of mycelium plus spores scraped from agar cultures was added into each vial and mixed with the soil. The screw caps were wrapped with Parafilm® to prevent dehydration. The tubes were stored at 5°C until use.

Sorghum seed samples: Naturally infected sorghum seeds from farmers own saved seed lots were chosen based on the pericarp colour (red and white) and on the incidence of *C. graminicola*, *P. sorghina* and *F. moniliforme* revealed by the standard blotter test performed at DSHC according to Mathur and Kongsdal (2003) (Table 1).

Botanicals and synthetic fungicide: Essential oils and crude oil were used in this study (Table 2). Essential oil (CEO) of lemongrass (*Cymbopogon citratus*), essential oil (EEO) of Eucalyptus (*Eucalyptus camaldulensis*) and crude oil (NCO) of neem (*Azadirachta indica*) were tested. The essential oils were prepared by the Research Institute for Applied Sciences and Technology (IRSAT) of Burkina Faso. Extraction was performed by using stream distillation. Throughout the study, the commercial fungicide Dithane M-45 (Zinc-manganese ethylene bisdithiocarbamate) (Dow chemicals) was used as positive control.

Testing effect of oil extracts on fungal growth: One isolate of each test fungal species was tested in three replicates for each concentration of Essential Oil (EO) using the agar diffusion method. Four concentrations of essential oils (2, 4, 6 and 8%) were tested. The oils were emulsified using 0.1% sterilized water agar (Remmal *et al*., 1993). Conidial suspensions of 10^6 conidia mL^{-1} were prepared from 7 days agar cultures. A drop of 50 μL of the suspension was streak on agar plates (9 cm diameter) containing 25 mL PDA. Ten microliters of the EO emulsion were loaded into each of the four wells (2 mm diameter) made in the agar medium. Control plates were loaded with either water agar (0.1%) or Dithane (0.3%,w/v). The plates were incubated at 25±1°C under cycles of 12 h NUV light/12 h darkness for 2-3 days. The Inhibition Zone (IZ) around each well was evaluated by measuring two perpendicular diameters for each well. The mean of diameters of the four wells gave the IZ for each replicate.

Seed treatment with oil extracts: The seeds were treated with an emulsion of essential oils (Eos) in 0.1% water agar

Table 1: Sorghum seed samples collected in south western region of Burkina Faso and their natural infection rates by pathogenic fungi

				Incidence of seed-borne fungi (%)		
Accession No.	Variety	Pericarp colour	Location	*C. graminicola*	*P. sorghina*	*F. moniliforme*
47056	Local	White	South/West	10.50	28.5	1.00
205/214	Local	Red	South/West	2.75	72.5	20.25

Table 2: Plant species collected from Ouagadougou, Burkina Faso environment and tested in this study

Plant species	Family name	Common name	Nature of the extracts	Place/year of harvest
C. citratus (D.C.) Stapf.	Gramineae	Lemongrass, Citronella	Leaf essential oil	Ouagadougou/2002
E. camaldulensis Dehnh.	Myrtaceae	Eucalyptus	Leaf essential oil	Ouagadougou/2002
A. indica A. Juss.	Meliaceae	Neem	Seed crude oil	Ouagadougou/2002

solution. Four concentrations were considered based on preliminary test (data not shown). The EO emulsions were tested at the concentrations 2, 4, 6 and 8%. One hundred microliters of the mixture were applied per gram of seeds in 50 mL-Erlenmeyer flasks according to Adegoke and Odesola (1996). Negative controls were treatment with water agar and untreated seeds. The positive control was treatment with Dithane M-45 at the rate of 0.3% of seed weight. After treatment, the flasks were covered with Parafilm®, shaken for 5 min and maintained overnight at 20-22°C in darkness.

Testing effect of oil extracts on seed health: Two hundred seeds were tested for each treatment. The oil-treated seeds were plated in Petri dishes containing three layers of moistened blotter papers according to Mathur and Kongsdal (2003). A set of four plates (100 seeds) was considered as replicate. Petri plates containing 25 seeds were considered as sub-replicates and randomly arranged in the incubation room. The positive control was treated with Dithane M-45 (0.3%, w/w). The negative control was treated with water agar. Set of untreated seeds was also considered. The disease incidence was recorded as percent of seed bearing a given fungus per replicate.

Testing effect of oil extracts on seedling emergence and growth: Two hundred seeds per treatment (100 seeds per replicate) were used for the growing-on test. Seeds were treated as described for the seed health experiment and were planted in standard peat soil (Weibull K-Soil) in plastic pots (14×13×6 cm), i.e., 25 seeds per pot and eight pots per treatment. After sowing, the pots were incubated in growth chamber at 28±2°C under cycles of 12 h fluorescent light/12 h darkness. Four pots per replicate were randomly arranged in the trays. After 3, 7 and 14 days, seedling emergence was evaluated and percentage of emerging seedlings calculated. After 14 days of incubation, the seedlings from each replicate were cut at the soil level and weighed.

Testing effect of oil extracts on fungal seed to seedling transmission: After weighing, 20 fresh plants randomly picked per replicate (i.e., five plants per pot) were assayed for recovery of the four seed-borne fungi cited above. The first leaves were removed and plated on moistened blotters. After surface disinfection and sterilization in 70% ethanol for 30 sec and in 1% sodium hypochloride for 1 min, respectively, stems were cut aseptically into smaller sections from the base up to ca 10 mm. These segments were plated on three layers of moistened blotter papers in Petri dishes and incubated at 25±1°C under NUV light (12 h/12 cycles). Stem and corresponding first leaf were plated and marked accordingly. After five days, the fragments were inspected under stereomicroscope for the presence or absence of the target fungi.

Data analyses: Treatment effects were determined by one-way analysis of variance using a completely randomised design. The significance of differences between treatments was determined, using the Least Significant Difference (LSD) test of Statgraphic statistical software, version 5. Pearson correlation was also performed to reveal the relationships between different parameters.

RESULTS

Effect of oil extracts on fungal radial growth: Isolates of *C. graminicola, P. sorghina* and *F. moniliforme* recovered from naturally infected seeds were tested *in vitro*. The results of this study showed differences in sensitivity of the different fungal species and in potency of essential oils. *C. citratus* essential oil exhibited antifungal activity against all the three species of fungi. *E. camaldulensis* essential oil showed very low level of antifungal activity and neem crude oil had no adverse effect on the growth of the test isolates (Table 3). *C. graminicola* was highly sensitive to essential oil of *C. citratus*; 76.2% inhibition was obtained using 8% emulsion. Fifty percent growth inhibition was recorded for *P. sorghina* at the highest concentration, while *F. moniliforme* was less sensitive to the lemongrass essential oil (21.4-41.3%). A dose dependent inhibition of *C. graminicola* mycelial growth was caused by lemongrass essential oil.

Effect of oil extracts on seed health: Statistical analyses showed significant effects of treatments on *C. graminicola* recorded on sample 47056 and on *P. sorghina* and *F. moniliforme* evaluated on sample 205/214. *C. citratus* essential oil was more effective than that of *E. camaldulensis* on *C. graminicola* (Table 4). The highest percent reduction of *C. graminicola* (94.4%) was recorded at the concentration 6%, while only 16.7% reduction was obtained with *E. camaldulensis* oil at concentration 8%. Essential oil from *C. citratus* was very effective against *P. sorghina* with reduction of infection percent ranging from 71.7-95.1%, while infection by *F. moniliforme* was reduced to levels ranging from 20.7-62.1% on sample 205/214. Both *P. sorghina* and *F. moniliforme* were less sensitive to *E. camaldulensis* essential oil (13.6 and 44.8% reduction of infection, respectively).

Table 3: Effect of oil extracts on mycelial growth inhibition of *Colletotrichum graminicola, Phoma sorghina* and *Fusarium moniliforme*[1]

	Fungal species tested		
Plant extracts[2]	*C. graminicola*	*P. sorghina*	*F. moniliforme*
A. indica			
100 (%)	0.0^{a} (0)	0.0^{a} (0)	0.0^{a} (0)
C. citratus			
2 (%)	5.1^{d} (27.6)	5.3^{cd} (31.2)	4.2^{b} (26.9)
4 (%)	9.0^{e} (48.6)	6.5^{d} (38.2)	4.9^{b} (31.4)
6 (%)	11.2^{f} (60.5)	8.1^{e} (47.6)	6.5^{c} (40.8)
8 (%)	14.1^{g} (76.2)	8.7^{e} (51.2)	8.1^{d} (51.9)
Dithane	18.5^{h} (100)	17.0^{f} (100)	19.6^{e} (100)
E. camaldulensis			
2 (%)	0.0^{a} (0)	0.0^{a} (0)	0.0^{a} (0)
4 (%)	0.0^{a} (0)	0.0^{a} (0)	0.0^{a} (0)
6 (%)	3.2^{b} (17.3)	3.3^{b} (19.4)	4.2^{b} (21.4)
8 (%)	4.2^{c} (22.7)	4.6^{bc} (27.1)	4.9^{b} (25.0)
Water agar	0.0^{a} (0)	0.0^{a} (0)	0.0^{a} (0)

[1]: Data are mean diameters (mm) of inhibitory zones round 12 wells per concentration, [2]: *Azadirachta indica crude* oil, essential oils of *Cymbopogon citratus* and *Eucalyptus camaldulensis* tested at different concentrations (v/v, oil/water agar). Mean values in the same column followed by the same letter are not significantly different. Data within brackets are the percent inhibition compared to treatment with Dithane M-45

Table 4: Effect of seed treatment with oil extracts on the incidence of *Colletotrichum graminicola, Phoma sorghina* and *Fusarium moniliforme*[1]

	Essential oil treatments[2]								Control		
	C. citratus (%)				*E. camaldulensus* (%)				WA	DIT	NT
Fungal species[3]	2	4	6	8	2	4	6	8	0.3 (%)		
Sample 47056											
C.g.	2.5 (72.2)^{bc}	3 (66.7)^{c}	0.5(94.4)^{ab}	1 (88.9)^{abc}	8 (11.0)^{d}	8 (11.0)^{d}	9 (0)^{d}	7.5 (16.7)^{d}	9 (0)^{d}	0 (100)^{a}	nt
P.s.	6.5	4	1	1	15	15	10	10	15	0	nt
F.m.	2.5	1	0.5	0	3	2.5	1.5	0.5	3	0	nt
Sample 205/214											
C.g.	3	3	3	1	1.5	1.5	2	1.5	0.5	0	1.5
P.s.	26 (71.7)^{d}	12 (87.0)^{bc}	18 (80.4)^{cd}	4.5 (95.1)^{ab}	91 (1.1)^{g}	90.5 (1.6)^{fg}	84.5 (8.2)^{efg}	79.5 (13.6)^{ef}	92 (0)^{g}	0 (100)^{a}	78.5^{e}
F.m.	11.5 (20.7)^{de}	7 (51.7)^{bc}	6.5 (55.2)^{bc}	5.5 (62.1)^{ab}	12 (17.2)^{de}	11 (24.1)^{de}	10 (31.0)^{cd}	8 (44.8)^{bcd}	14.5 (0)^{e}	1.5 (89.7)^{a}	9.5^{cd}

[1]: Data are mean N. of infected seeds per replicate, [2]: Essential oils tested at four different concentrations (v/v), [3]: C.g. = *C. graminicola*, P. s. = *P. sorghina*, F. m. = *F. moniliforme*; Data within brackets are the percent reduction compared to negative control (WA); WA: water agar, DIT: Dithane-M45 used at dosage 0.3% (w/w), NT: non-treated, nt : not tested. Mean values followed by the same letter are not significantly different ($p<0.05$)

Effect of oil extracts on fungal seed to seedling transmission: Treatment effects on transmission of the target fungi from seeds to leaves and stems were not statistically significant. Therefore, Fig. 1 describe the trend of the seedling infection compared to percent infection of seed. The transmission rate was higher than expected, based on the results from the seed health testing. On sample 47056, having the highest initial incidence of *C. graminicola* (10.5%), leaf and stem transmissions were observed with percent infection more than 10% (Fig. 1a). *C. citratus* essential oil reduced interestingly the transmission of *C. graminicola* and *P. sorghina*, as compared to the water agar control (Fig. 1a, b). *E. camaldulensis* had no adverse effect on the transmission of the target fungi (Fig. 1). As shown in Fig. 1b, *P. sorghina* was less transmitted to both leaves and stems, while *F. moniliforme* appeared highly transmitted to seedlings of both samples tested (Fig. 1c, d). The synthetic fungicide Dithane M-45 also appeared less effective against *F. moniliforme* on sample 205/214 (Fig. 1d). On sample 205/214 exhibiting 20.25% seed infection in the original sample, there was no apparent difference in seed-to-seedling transmission of *F. moniliforme* after treatments.

Effect of oil extracts on seedling emergence: A highly significant treatment effect on seedling emergence was found at the different dates of evaluation (Fig. 2). All the treatments were compared to the water agar control for both seed samples (47056 and 205/214) displaying potential emergence of 84 and 82%, respectively. Neem crude oil showed high inhibitory effect on seed germination and emergence on both samples. Emergence of 40% was hardly obtained with neem oil across the sample and the dates of evaluation (Fig. 2). Essential oil from *C. citratus* showed a clear depressive effect on sample 47056 when high concentrations were used (Fig. 2a). Lower concentrations (2 and 4%) were as good as the negative control (*ca* 100% emergence). Emergence was 10% higher with Dithane treatment than that of the negative control (Fig. 2a). The same trend was observed with sample 205/214 Fig. 2c). The highest concentration of *C. citratus* essential oil (8%) was markedly harmful to emergence. Interestingly, concentration of 6% was as effective as 2 and 4% in having no depressive effect on seedling growth. In return, essential oil from *E. camaldulensis* was slightly more effective than the control with percent emergence

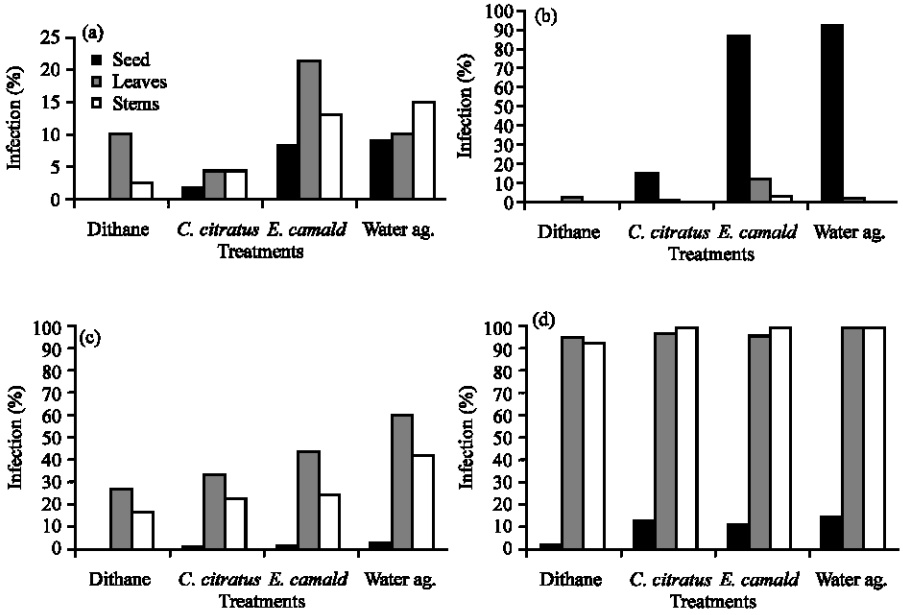

Fig. 1: Effect of essential oils on seed infection and seed to seedling transmission of *C. graminicola*, on sample 47056 (a), *P. sorghina* on sample 205/214 (b) *F. moniliforme* on samples 47056 and 205/214, respectively (c, d)

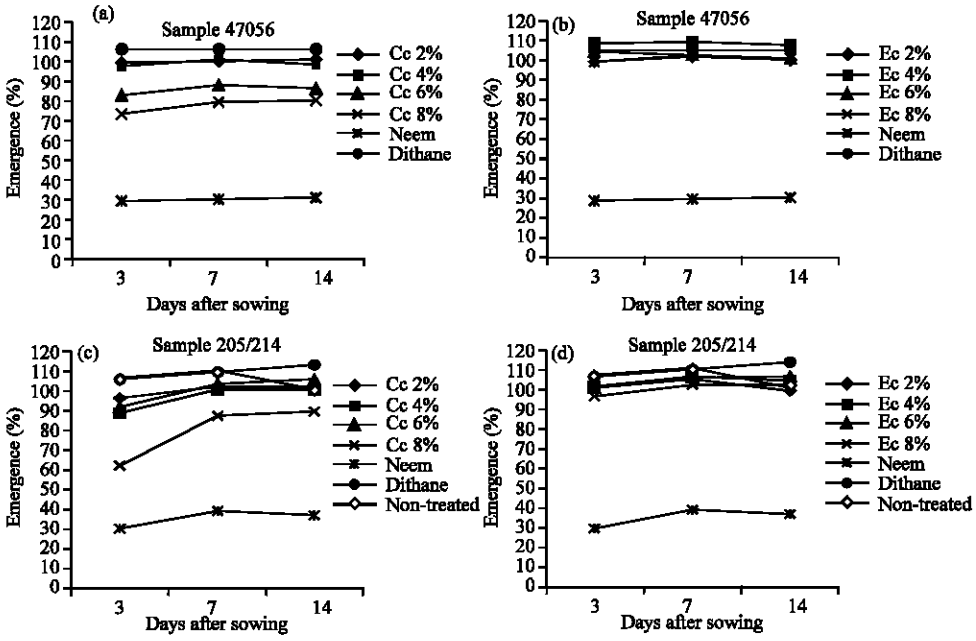

Fig. 2: Effect of treatment with essential oils of *Cymbopogon citratus* (Cc) (a, c), *Eucalyptus camaldulensis* (Ec) (b, d) at four concentrations and neem crude oil on the emergence percentage of two sorghum samples (47056 and 205/214) compared to water agar control (potential emergence of the samples 47056 and 205/214 are 84 and 82%, respectively)

ranging from 100-110% on the white sorghum sample 47056 (Fig. 2b). Similarly, on the red sorghum sample 205/214, the effects of the different concentrations used were comparable to the controls. On both samples, concentrations of *E. camaldulensis* essential oils had no adverse effect on seedling emergence which percentages ranged from 95-110% compared to the negative control (Fig. 2b, d).

Table 5: Effect of plant extracts on sorghum seedling growth rate (nb day^{-1}) and total biomass (g)[2]

Plant Extracts[3]	Sample 47056		Sample 205/214	
	Growth rate	Total biomass	Growth rate	Total biomass
A. indica				
100 (%)	3.6^{a}	5.6^{a}	4.5^{a}	7.0^{a}
C. citratus				
2 (%)	11.8^{cd}	20.7^{bc}	11.9^{cd}	23.0^{cd}
4 (%)	11.9^{d}	20.0^{bc}	11.6^{c}	21.9^{bcd}
6 (%)	10.4^{bc}	20.2^{bc}	12.0^{cde}	24.7^{de}
8 (%)	9.4^{b}	18.1^{b}	10.1^{b}	18.5^{b}
Dithane M-45	12.5^{d}	26.8^{e}	12.7^{de}	27.2^{e}
E. camaldulensis				
2 (%)	12.1^{d}	17.8^{b}	12.1^{cde}	21.4^{bcd}
4 (%)	12.9^{d}	25.7^{bc}	12.3^{cde}	23.4^{bcd}
6 (%)	12.2^{d}	22.6^{bc}	12.2^{cde}	24.3^{de}
8 (%)	12.1^{d}	24.0^{bc}	11.8^{c}	21.9^{bcd}
Water agar	11.9^{d}	26.1^{bc}	11.6^{c}	21.8^{bcd}
Non treated	nt	nt	12.8^{e}	20.6^{bc}

[1]: Data are mean numbers of seedlings per replicate; [2]: Data are mean fresh weights of seedlings per replicate; [3]: *A.. indica crude* oil, essential oils of *Cymbopogon citratus* and *Eucalyptus camaldulensis* tested at different concentrations (v/v). Mean values in the same column followed by the same letter are not significantly different ($p<0.05$)

Table 6: Relationships between fungal radial growth inhibition seed health and seed to seedling transmission of *Colletotrichum graminicola* (a), *Phoma sorghina* (b) and *Fusarium moniliforme* (c) after treatment with essential oils

Parameters	Seed infection	Transmission	
		Stem	Leaf
Sample 47056			
(a) *C. graminicola*			
Fungal growth inhibition[1]	-0.85**	NS	NS
Seed infection		0.59*	NS
Stem transmission			0.74*
Sample 205/214			
(b) *P. sorghina*			
Fungal growth inhibition	-0.93**	NS	NS
Seed infection		NS	NS
Stem transmission			0.59*
(c) *F. moniliforme*			
Fungal growth inhibition	-0.76*	-0.86**	NS
Seed infection		0.68*	NS
Stem transmission			NS

[1]: Inhibition zones round the wells containing the antagonist compound tested. Values are Pearson product moment correlation coefficients followed by *: $p<0.05$, significant, **: $p<0.01$, highly significant, NS: Non significant

Effect of oil extracts on seedling growth rate and biomass production: Neem oil and both essential oils significantly affected seedling growth rate and total biomass (Table 5). Neem crude oil exhibited the greatest inhibitory effect on growth rate (3.6 and 4.5 seedlings day^{-1} for samples 47056 and 205/214, respectively and biomass (5.6 and 7.0 g for samples 47056 and 205/214, respectively. *C. citratus* essential oil at concentration of 8% significantly reduced both growth rate and total biomass, whereas *E. camaldulensis* essential oil had no depressive effect (Table 5).

Relationships between inhibition of fungal radial growth, seed health and seed transmission: Since essential oils showed effects on different parameters, the influence between those parameters was evaluated (Table 6). The inhibitory effect of essential oil treatments on fungal radial growth was highly and negatively correlated to the incidence of target fungi on seed; correlation coefficients ranging from -0.76 to -0.93. The transmission of *C. graminicola* from seed to stems was weakly and positively correlated to seed infection (0.59). No significant correlation was found between seed infection of *P. sorghina* and its seed to seedling transmission. There were positive and low relationships between stem and leaf transmission of *C. graminicola* and *P. sorghina*. Mycelial growth inhibition *in vitro* and seed to stem transmission of *F. moniliforme* were negatively and highly correlated (-0.86), while weak and positive correlation was found between seed infection and stem transmission (0.68).

DISCUSSION

Phoma sorghina and *Fusarium moniliforme* are responsible of grain mould on sorghum grown in Burkina Faso. Furthermore, *Colletotrichum graminicola* is the most threatening grain, leaf and stalk disease of sorghum in smallholder farmers fields. These fungi were the main pathogens encountered in many seed samples from Burkina Faso analysed at the Danish Government Institute of Seed Pathology for Developing Countries, Denmark (Somda *et al*., unpublished data). Extracts from local plants (botanicals) may provide resource-poor farmers with an option to control seed-borne pathogens of their own saved seeds, using locally available, environmentally friendly methods. The overall aim sought in this study was to evaluate the efficacy of essential and crude oils in controlling major facultative pathogens carried by sorghum seeds without inhibiting seed germination and seedling growth. The agar diffusion test showed that essential oil emulsions exhibit an inhibitory property against *C. graminicola*, *P. sorghina* and *F. moniliforme*. Neem crude oil had no effect on the target fungi. This contrasts with the results of Amadioha (2000), who found radial growth reduction of *Pyricularia oryzae* Cav. by neem oil extracts. The lack of inhibitory activity may be a result of low concentration or lack of active constituents, or simply because of the oil extraction method. Nevertheless, results of the radial growth experiments showed that this modified agar diffusion method was efficient in screening low volumes of essential oils. *C. citratus* essential oil showed the highest antifungal activity against *C. graminicola, P. sorghina* and *F. moniliforme*, as compared to the fungicide Dithane M-45. *E. camaldulensis* essential oils were slightly effective at high concentrations. These results showed that the inhibitory effect of *C. citratus* essential oil was

concentration dependent. Interestingly, strong relations were obtained between the inhibition of fungal radial growth by essential oils and their effects on seed infection along with seedling growth regulation. This result infers that the agar diffusion test can actually be used as a first step when evaluating the potency of plant extracts to control seed-borne pathogenic organisms. Another important point raised by present study is that essential oils were effective in controlling the test fungi on sorghum seeds. Of the three plants extracts evaluated, *C. citratus* essential oil was the most potent. The concentrations of 6 and 8% caused the greatest reduction of seed infection at levels comparable to the fungicide Dithane M-45. *E. camaldulensis* essential oils were inferior as fongitoxicant on sorghum seeds, confirming its lack of potency demonstrated *in vitro*. Studies on the uses of plant extracts to control grain spoilage and phytopathogenic fungi are well documented (Dubey *et al*., 2000; Bajwa *et al*., 2003; Aladi *et al*., 2005; Yulia *et al*., 2006; Cardenas-Ortega *et al*., 2007). The fungitoxic effects of lemongrass essential oil were in agreement with the results obtained by Banjole and Joda (2004), Nguefack *et al*. (2004) and Abd-El-Khair and Wafaa (2007). As seed treatment, lemongrass essential oil at concentrations 6 and 8% was more effective against *C. graminicola* in reducing seed infection by 66.7 to 94.4% in the white sample (47056) and by 71.7 to 95.1% in the red sorghum sample 205/214. More than 50% of the growth of *F. moniliforme* was reduced by *C. citratus* essential oil both *in vitro* and *in vivo* on seed whereas *E. camaldulensis* essential oil was less efficient even at high concentrations. The weaker control effect obtained with *E. camaldulensis* essential oil against all tested fungi could be attributed to its contents of known antimicrobial compounds. *C. citratus* from Burkina Faso contained citral a and b (Menut *et al*., 2000), which is absent in *E. camaldulensis* essential oils (Maximous, 2004).

The potential use of plant oil extracts for the control of plant diseases requires identification of extracts which inhibit the growth of pathogens at non-phytotoxic concentrations. *C. citratus* essential oil was efficient in reducing seedling infection of *C. graminicola*, *P. sorghina* and, in some instances, *F. moniliforme* at levels comparable to the fungicide Dithane M-45. Low concentrations (2 and 4%) of *C. citratus* essential oil improved seedling emergence, while high concentrations maintained seedling emergence at a level equivalent to the potential emergence of the original sorghum seed samples.

CONCLUSION

The present study concludes that lemongrass essential oil at 6% concentration can be effectively used to treat sorghum seed against pathogenic fungi, namely *C. graminicola* and *P. sorghina*. These findings prompt further investigations on the effectiveness of this oil extract under field conditions.

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