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# Evaluation of Lemongrass, Eucalyptus and Neem Aqueous Extracts for Controlling Seed-borne Fungi of Sorghum Grown in Burkina Faso

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**Abstract:** Aqueous extracts of *Cymbopogon citratus* (lemongrass), *Eucalyptus camaldulensis* (eucalyptus) and *Azadirachta indica* (neem) were tested for inhibitory activity against *Colletotrichum graminicola*, *Phoma sorghina* and *Fusarium moniliforme* in naturally infected sorghum seeds. Two seed samples (red and white cultivars) exhibiting different rates of infection by the target fungi were treated with two concentrations of each plant aqueous extract (15 and 30% (w/v)). Moist blotter paper and growing-on tests were performed to evaluate the efficacy of botanicals to control fungi on sorghum seeds and seedlings. Aqueous extract of *C. citratus* exhibited the best control effect on seed infection by *C. graminicola* and *P. sorghina* and subsequent seed to seedling transmission of *C. graminicola*. Infections by *C. graminicola* and *P. sorghina* were significantly reduced by more than 60% when using 15% concentration and by 100% when using 30% concentration of lemongrass extract. Lemongrass extract did not affect the seedling development. Eucalyptus and neem seed aqueous extracts were not effective in controlling seed infection and seed transmission of the test fungi, but increased seedling emergence. Lemongrass extract has potential as sorghum seed disinfectant against *C. graminicola* and *P. sorghina*.

**Key words:** Aqueous extract · seed-borne fungi · seed transmission · seed treatment · sorghum

## INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most commonly cultivated crop in Burkina Faso with production estimated to 48% of the cereals grown in 2004, covering 44% of the area harvested [6]. An average annual production during the 2000-2004 period was 1.4 million tons, with low yield (0.9 ton $ha^{-1}$) [6]. Sorghum cultivation is impeded by various abiotic and biotic constraints [10, 14]. Many fungal diseases reported on sorghum are mainly seed transmitted [15] and are built up in the soil. Sorghum anthracnose caused by *Colletotrichum graminicola* (Ces.) Wilson (Syn. *C. sublineolum* P. Henn) is one of the most important seed-borne disease in Burkina Faso [11]. The fungus is responsible of leaf, stalk and grain anthracnose as well as head mould. Combination of leaf and stalk infection can cause yield losses up to 46% [10]. Of importance is also *Fusarium moniliforme* Sheldon (Syn. *F. thapsinum* Klittich, Leslie, Nelson et Marasas sp. nov.) [7] which causes seed and seedling foot rots and, is involved in the head mould complex along with *Phoma sorghina* (Sacc.) Boerema, Dorebosch and van Kesteren. [14]. Breeding for genetic resistance and use of fungicides have been the most effective strategies to control sorghum diseases but quality seed of improved varieties does not reach traditional small-scale farmers who use their own-saved seeds without chemical treatment. Synthetic fungicides are not always available or affordable by subsistence farmers to improve the quality of their seeds and to protect their crops. Lack of control measures available to smallholder farmers and problems associated with the use of synthetic pesticides (resistance, persistence, toxicity, restriction of use, etc.) have prompted researches on the

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use of environmentally safe methods to control pathogens. Investigations on local botanicals which might contribute to the development of new alternative control measures are extremely important in the context of Burkina Faso agriculture. Use of plant parts or derivative such as wood-ash, to control insect pests of stored products and backyard vegetables has been practised in Africa [13]. Plants such as neem, thyme, lemongrass as well as many others have been successfully used to control important seed-borne fungi [2, 8]. In Nigeria, lemongrass powder and essential oil have been successfully used to protect cowpea and maize against storage fungi and *Macrophomina phaseolina* (Tassi) Goid. [1]. Aqueous extract of lemongrass was also effective against seed-borne fungal pathogens of melon [4]. More recently, Nteso and Pretorius [12] reported that seed treatment with *Tulbaghia violacea* water extract significantly reduced the incidence of both sorghum loose and covered smut diseases. Despite the many studies performed on plant extracts, relatively little is known about their role in controlling seed-borne facultative pathogens which are the main constraints of sorghum production in Burkina Faso. Therefore, our study aimed at (1) evaluating the potential of selected plant extracts in controlling seed-borne pathogens of sorghum, (2) studying the effect of plant extract on seed vigour and seed-to-seedling transmission of some pathogenic fungi.

## MATERIALS AND METHODS

**Botanicals and synthetic fungicide:** Characteristics of plants extracts tested in this study are reported in Table 2. Leaf powder (CLP) of lemongrass (*Cymbopogon citratus* (D.C.) Stapf.), leaf powder (ELP) of Eucalyptus (*Eucalyptus camaldulensis* Dehnh.), seed powder (NSP) and leaf powder (NLP) of neem (*Azadirachta indica* A. Juss.) were tested. Throughout the study, the commercial fungicide Dithane M-45 (Zinc-manganese ethylene bisdithiocarbamate) (Dow chemicals) was used as positive control.

**Preparation of plant aqueous extracts:** Aqueous extracts were obtained by mixing dry powdered plant parts with deionised water at the given rate (w/v) and leaving the mixture overnight at 20-22°C. The mixtures were then filtered though four folds of cheesecloth followed by sterile-filtering through a 0.2 μm filter (Millipore Express™ PLUS), before immediate use. For each aqueous extract, 15 and 30% concentrations (w/v) were studied.

**Seed treatment with aqueous extracts:** Two (2) concentrations of plant aqueous extracts (15 and 30% (w/v)) were freshly prepared. Two hundred (200) seeds per treatment were soaked in 20 ml of the test aqueous extract in 50 ml-Erlenmeyer flasks covered with Parafilm®. The flasks were kept overnight at 20-22°C in darkness. Before subsequent testing, seeds were collected on cheese cloth and air-dried.

**Testing effect of aqueous extracts on seed health:**

deionised water, respectively. A set of untreated seeds was also considered as control. The disease incidence was recorded as percent of seeds bearing a given target fungus per replicate.

**Testing effect of aqueous extracts on seedling emergence and growth:** Two hundred (200) seeds per treatment (100 seeds per replicate) were used for the growing-on test. Seeds were treated as described above and planted in standard peat soil (Weibull K-Soil) in plastic pots (14×13×6 cm), i.e. 25 seeds per pot and 8 pots per treatment. After sowing, the pots were incubated in growth chamber at 28±2°C and 12 h light provided by white fluorescent lamps. The 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, seedlings from each replicate were cut at the soil level and weighed.

**Testing effect of aqueous extracts on seed-to-seedling transmission of fungal pathogens:** Twenty (20) fresh plants randomly picked per replicate (i.e. five plants per pot) were assayed for recovery of *Colletotrichum graminicola, Fusarium moniliforme* and *Phoma sorghina*. The first leaves were collected and stems were surface-sterilized (30 s in 70% ethanol and 1 min in 1% sodium hypochloride) and cut aseptically into smaller sections from the base up to ca 10 mm. Leaf and stem segments were plated on three layers of moistened blotter papers in Petri dishes and incubated at 25±1°C under NUV (12 h/12 cycles). After five days, the plant parts were inspected under stereomicroscope for the presence or absence of the target fungi.

**Data analyses:** Treatment effect was determined by one-way analysis of variance (ANOVA) using a completely randomised design. The significance ($p<0.05$) of differences between treatments was determined, using the LSD test of Statgraphic, version 5.

## RESULTS

**Effect of aqueous extracts on seed health:** Treatment effect was statistically significant, except for *Fusarium moniliforme* on sample 47056 which exhibited 1% infection in original sample (Table 3). *Cymbopogon citratus* extract showed a total control of *Colletotrichum graminicola* and *Phoma sorghina* on sorghum seed sample 47056 at 30% concentration. Similarly, *C. graminicola* was completely controlled on sample 205/214, while *P. sorghina* was reduced to 65.7% compared to the negative control. *Eucalyptus camaldulensis* aqueous extract showed slight effects in reducing seed-borne infection by *P. sorghina* on sample 205/214 (29.5 and 32.4%). *Azadirachta indica* leaf and

Table 3: The effects of seed treatment with aqueous extracts on the incidence of *Colletotrichum graminicola*, *Phoma sorghina* and *Fusarium moniliforme*[1]

| | Aqueous extracts[2] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Cymbopogon citratus* | | *Eucalyptus camaldulensis* | | *Azadirachta indica* leaf | | *Azadirachta indica* seed | | Controls | | |
| Fungal species | 15% | 30% | 15% | 30% | 15% | 30% | 15% | 30% | DW | Dithane 0.3% | NT |
| Sample 47056 | | | | | | | | | | | |
| *C. graminicola* | 4ab | 0a | 7ab | 6ab | 7ab | 5.5ab | 9.5b | 7.5ab | 10.5b | 0a | 10b |
| | (61.9) | (100) | (33.3) | (42.9) | (33.3) | (47.6) | (9.5) | (28.5) | (0) | (100) | |
| *P. sorghina* | 5.5ab | 0a | 7abc | 7abc | 6.5abc | 9bc | 7.5abc | 6abc | 14c | 0a | 11.5bc |
| | (60.7) | (100) | (50) | (50) | (53.6) | (35.7) | (46.4) | (57.1) | (0) | (100) | |
| *F. moniliforme* | 0 | 0 | 1 | 0.5 | 1 | 2 | 0.5 | 2 | 0.4 | 0.5 | 0.6 |
| Sample 205/214 | | | | | | | | | | | |
| *C. graminicola* | 1ab | 0a | 3.5bcd | 4bcd | 2bcd | 2bcd | 3.5bcd | 2.5bcd | 3.5bcd1ab | 5.5d | (71.4) |
| | (100) | (0) | (14.3) | (42.9) | (42.9) | (0) | (-28.6) | 0 | (71.4) | | |
| *P. sorghina* | 35c | 18b | 35.5c | 37c | 85.5ef | 77e | 92.5f | 94.5f | 52.5d | 1a | 56.5d |
| | (33.3) | (65.7) | (32.4) | (29.5) | (-62.9) | (-46.7) | (-76.2) | (-80.0) | 0 | (98.1) | |
| *F. moniliforme* | 11ab | 10ab | 14bcd | 16bcd | 15.5bcd | 23.5d | 13.5bc | 22.5cd | 11.5ab2.5a | | 19bcd |
| | 4.3 | (13) | (-21.7) | (-39.1) | (-34.8) | (-104.3) | (-17.4) | (-95.7) | (0) | -78.3 | |

[1]:Data are mean numbers of infected seeds per replicate, [2]:Aqueous extracts tested at two different concentrations (w/v); (): Percent reduction compared to negative control (DW); DW: Deionised water, DIT: Dithane-M45 used at dosage 0.3% (w/w), NT: Non-treated. Mean values followed by the same letter are not significantly different ($p<0.05$)

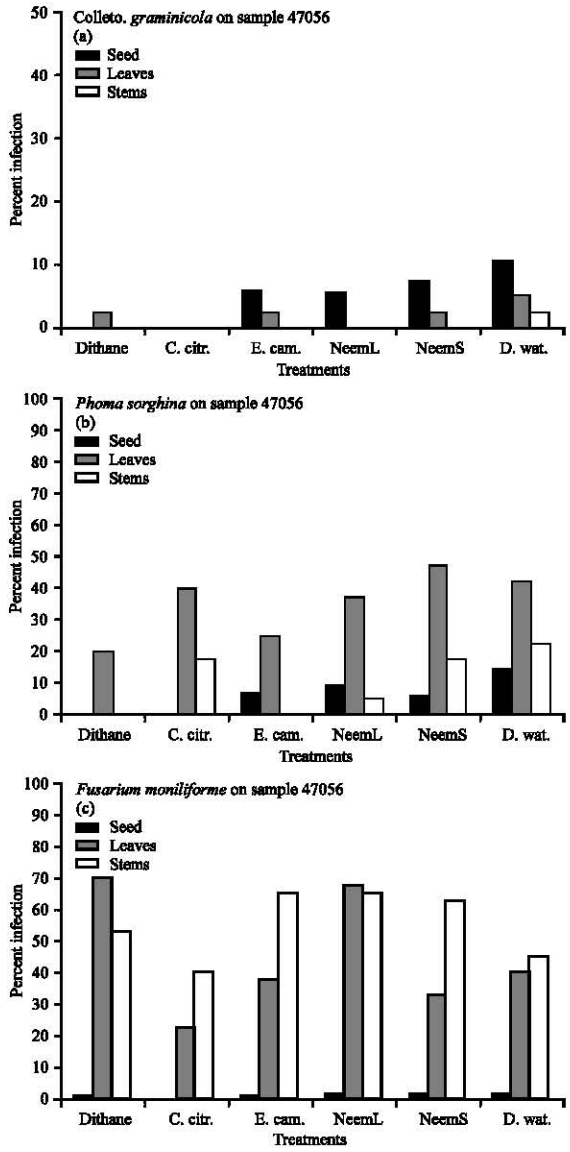


Fig. 1: The effect of aqueous extracts on seed health and seed to seedling transmission of *C. graminicola*, (a), *P. sorghina* (b) and *F. moniliforme* (c) (data are computed across the concentrations)

seed extracts were not efficient in reducing fungal seed infection. Apart from extract of *C. citratus*, other plant extracts had the tendency to increase seed infection by *F. moniliforme* on sample 205/214 (Table 3).

**Effect of aqueous extracts on seed-to-seedling transmission of fungal pathogens:** The effects of aqueous extracts on seed transmission are illustrated in Fig. 1. Aqueous extracts had different antifungal activities. The most efficient extract was that of *C. citratus*

Table 4: The effect of plant extracts on sorghum seedling growth rate (nb/day)[1] and total biomass (g)[2]

| | Sample 47056 | |
|---|---|---|
| Plant extracts[3] | Growth rate | Total biomass |
| Aqueous extracts | | |
| CLP 15% | 12.6 | 35.4 |
| ELP 15% | 12.9 | 40.4 |
| NLP 15% | 12.2 | 34.4 |
| NSP 15% | 12.5 | 32.9 |
| Deionized water | 13.1 | 39.3 |
| CLP 30% | 12.2 | 35.9 |
| ELP 30% | 13.0 | 38.6 |
| NLP 30% | 12.3 | 33.6 |
| NSP 30% | 12.1 | 40.9 |
| Dithane M-45 | 12.4 | 32.2 |
| Non treated | 12.1 | 29.0 |

[1]Data are mean numbers of seedlings per replicate; [2]Data are mean fresh weights of seedlings per replicate; [3]Leaf aqueous extracts of *C. citratus* (CLP), *E. camaldulensis* (ELP), *A. indica* (NLP) and seed aqueous extracts of *A. indica* (NSP) were tested at two different concentrations (w/v)

against *C. graminicola* (Fig. 1a). In general, high leaf infection by *P. sorghina* was obtained regardless the treatment used (Fig. 1b). Except for *C. citratus*, seed treatment with other plant extracts and the fungicide Dithane M-45 was less effective in controlling seed-to-seedling transmission of *F. moniliforme* (Fig. 1c).

**Effect of plant extracts on seedling emergence:** Aqueous extracts tested influenced sorghum seedling emergence (Fig. 2). Treatments were compared to the non-treated control of sample 47056 with a potential of 84% of seedling emergence. Surprisingly, the deionised water control generally increased seedling emergence as compared to other treatments, including Dithane M-45. Seedling emergence recorded at 3 days after sowing was enhanced by aqueous extracts, as compared to the fungicide treatment (Fig. 2). Lemongrass and neem seed aqueous extracts slightly inhibited seedling emergence at 30% concentration, as compared to 15% concentration (Fig. 2a and 2b). *E. camaldulensis* extract highly improved emergence; the concentration of 30% being the most effective (Fig. 2c). Effect of neem leaf extract was comparable to that of Dithane M-45 at 7 and 14 days after sowing (Fig. 2d).

**Effect of plant extracts on seedling growth rate and biomass production:** Data of seedling growth rate and total biomass evaluated using the sorghum seed sample

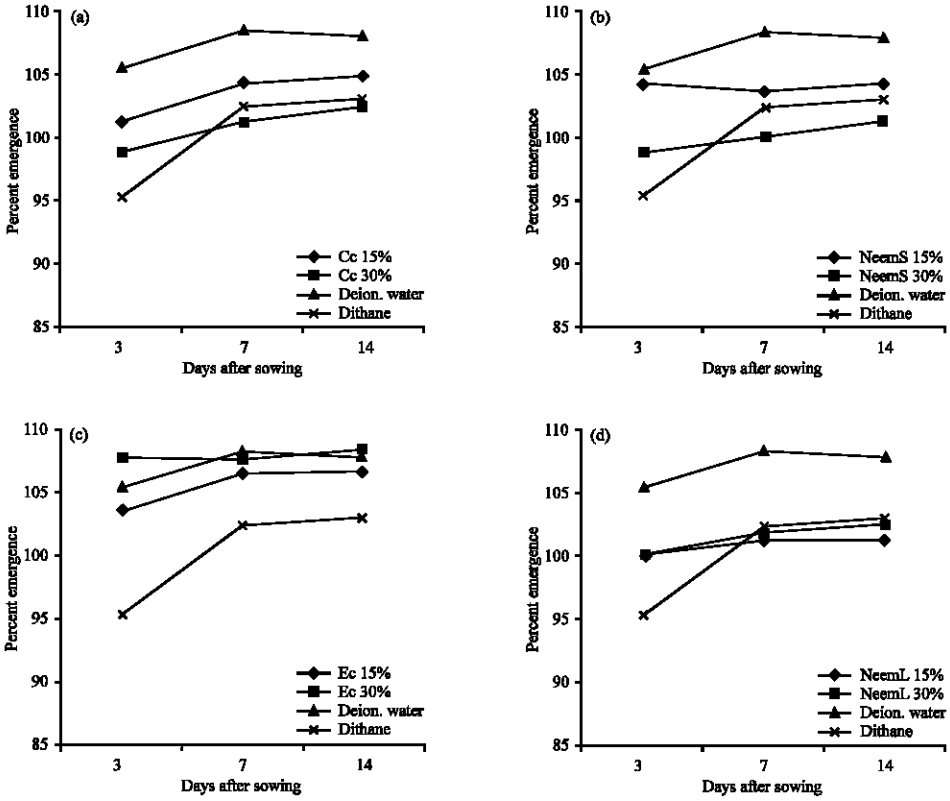


Fig. 2: The effect of treatment with aqueous extracts of *Cymbopogon citratus* (Cc) (a), neem seeds (NeemS) (b), *Eucalyptus camaldulensis* (Ec) (c), neem leaves (NeemL) (d) at two concentrations on the emergence percentage of sorghum (sample 47056) compared to the non-treated control (potential emergence of the sample : 84 %)

47056 are presented in Table 4. No significant effect was obtained. Aqueous extracts tested were not harmful to seedling growth and biomass production.

## DISCUSSION

The aim of this study was to evaluate the efficacy of botanicals in controlling three facultative fungal pathogens of sorghum carried by seeds without inhibiting seed germination and seedling growth. Aqueous extracts of lemongrass leaves at 30% concentration, showed potency in controlling seed infection by *Phoma sorghina* and *Colletotrichum graminicola* with reduction percentage of infection ranging from 65.7 to 100%, respectively. Similar results were obtained by Bankole and Adebanjo [4] and Amadioha and Obi [3]. Aqueous extracts of *C. citratus* completely reduced melon seed infection by *Macrophomina phaseolina*, *F. moniliforme* and *Botryodiplodia theobromae* Pat. [4]. Cold water extract of *C. citratus* checked the spread of anthracnose disease of cowpea *in vivo* [3]. Compared to white sorghum seed sample, neem aqueous extract increased seed infection by *F. moniliforme* and *P. sorghina* on the red sorghum seed sample exhibiting the original infection rate of 72.5%. These results are consistent with those of Conventry and Allan [5] who reported an increase of the growth of *Gaeumannomyces graminis* (Sacc.) Arx and Oliv. var. *tritici* Walker by neem seed extract. The concentration of the inhibitory compounds may have been lower than their effective dose; situation conducing to the stimulation of fungal growth [5]. However, differences in control effects on both types of sorghum seeds could be related to the composition of sorghum seed pericarp and testa [16]. Seed pericarp, testa colour and their components may play a role in the adhesion or absorbance of the inhibitory compounds soluble in aqueous extract of neem seed. The potential use of aqueous extracts to control plant diseases requires the identification of extracts which inhibit the growth of the pathogens at non-phytotoxic concentrations. Lemongrass leaf aqueous extract was as efficient as Dithane M-45 in controlling seed-to-seedling transmission of *C.*

*graminicola*. Moreover, this extract increased seedling emergence at levels comparable to Dithane M-45. Results of the current work have clearly shown that *C. citratus* (lemongrass) aqueous extract has potential to control anthracnose fungus (*C. graminicola*) and *P. sorghina*. Extracts from lemongrass may provide resource-poor farmers with an option to control seed-borne fungi of their own-saved seeds using locally available, environmentally-friendly methods. Nevertheless, efficacy of lemongrass aqueous extract should be investigated under field conditions and the active constituents should be determined and quantified.

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