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Effect of Aqueous Extracts of *Acacia gourmaensis* A. Chev and *Eclipta alba* (L.) Hassk. on Seed Health, Seedling Vigour and Grain Yield of Sorghum and Pearl Millet

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Abstract: Antifungal activity against several fungi infecting sorghum and pearl millet seeds was investigated in aqueous extracts of *Acacia gourmaensis* (bark) and *Eclipta alba* (whole plant). The effect of plant extracts in the management of the fungi *Fusarium moniliforme*, *Curvularia lunata*, *Phoma sorghina*, *Colletotrichum graminicola* and *Exserohilum rostratum* was assessed using the following parameters: post-treatment seed infection rate, emergence, mortality and vigour of seedlings and grain yield. Both plant extracts were efficient in controlling *P. sorghina*, *F. moniliforme* and *C. lunata* in pearl millet seeds where infections were reduced by 56-86%. In sorghum seeds, both extracts also reduced *P. sorghina* infections by 27-72% but only extracts from *A. gourmaensis* controlled *C. graminicola* with 69% decrease in seed infection. In addition to promoting seed health, all plants extracts favoured seedling emergence, especially in sorghum where proportions of emerged seeds (70-80%) were significantly higher than that of non-treated seeds (66%). Higher seedling vigour was also induced upon seed treatments in either sorghum (7.1-8.3) or pearl millet (7.4-8.4) compared to non-treated seeds (4.3 and 6.3, respectively). The overall beneficial effects of seed treatments with plant extracts resulted in the increase in grain yield. Treatment of the seeds with extracts from *E. alba* led to the highest yields in both sorghum (2.5 vs. 1.5 t ha^{-1} for non-treated seeds) and pearl millet (1.6 vs. 1.3 t ha^{-1}). Altogether, plant extracts from *A. gourmaensis* and *E. alba* showed fungicidal activities and may be used for controlling major sorghum and pearl millet seed-borne fungi.

Key words: Botanicals, antifungal activity, seed-borne fungi, vigour promoting

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) and pearl millet (*Pennisetum glaucum* (L.) R. Br.) are two staple cereal crops in Burkina Faso. They are grown on up to 2.6 millions hectares, which represents 88% of total under cultivation of cereal crops. According to the Food and Agriculture Organization (Faostat online: www.fao.org), corresponding grain production in 2006 was estimated at 2.1 millions tons (79% of the country cereal production). Although sorghum and pearl millet are socio-economically important, their cultivation is affected by various constraints which are responsible

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for low productivity. Average yields are estimated at 830 and 720 kg ha^{-1}, respectively for sorghum and millet. Recently, many fungi affecting seed germination or causing pre- or post-emergence rotting, seedling blight and head mould were reported as major biotic constraints for both sorghum and pearl millet (Mathur and Manandhar, 2003; Somda *et al*., 2006; Zida *et al*., 2008). Associated fungi species belonged to the following genera: *Colletotrichum* Corda, *Fusarium* Link, *Curvularia* Boedijn, *Phoma* Sacc. and *Exserohilum* Leonard and Suggs. Moreover, fungal infections in seeds were found to be high as incidence varied between 25 and 100% (Zida *et al*., 2008). In earlier studies, *Colletotrichum graminicola* which causes anthracnose as well as head mould was found to be responsible for yields losses reaching 46% in sorghum (Néya and Kaboré, 1987).

The use of chemicals to control seed-borne fungal diseases in sorghum and pearl millet is considered to be the most effective strategy. But, chemicals are not always available and they are often costly for the small-scale farmers. Moreover, repeated use of synthetic pesticides can lead to induction of genetic resistance in target organisms as it was demonstrated for carboxine used to control *Ustillago nuda*, the causal agent of wheat smut (Leroux, 1987).

In many instances, plant extracts have been used as effective and cheaper alternatives to chemicals in combatting insect pests and fungal diseases of plants (Koumaglo *et al*., 1998; Djibo, 2000; Nébié, 2006; Somda *et al*., 2007a, b; Zida *et al*., 2008). Ekpo and Benjoko (1994) reported that ash from *Azadirachta indica*, *Vernonia amygdaline*, *Gliricidia sepium* and *Cassia siamea* protected efficiently maize seeds against *Curvularia lunata*. Extracts of *Azadirachta indica* were also found to be effective in controlling *Alternaria alternata*, a seed-borne pathogen of wheat which affects both grain and flour quality (Hannan *et al*., 2005). Other plants whose extracts had similar effects were *Lawsonia inermis* and *Datura stramonium*. Furthermore, *in vitro* growths of *A. alternata* and of two other seed-borne fungi (*Drechslera hawaiiensis* and *D. tetramera*) were found to be inhibited by aqueous extracts of *Eucalyptus* spp. (Bajwa and Iftikhar, 2005).

In spite of their advantages of plants extracts in controlling pests and diseases in agriculture, plant potential in this field is still insufficiently exploited. In many countries, efforts are been devoted to studies that can promote the use of plants extracts to control pests and diseases. The objective of the present study was to investigate the use of aqueous extracts from *Acacia gourmaensis* and *Eclipta alba* for their potential effect in controlling major seed-borne fungi of sorghum and pearl millet and improving grain yield at farm level. Traditionally, both plants are used by farmers in trying to control some plant pathogens.

MATERIALS AND METHODS

Preparation of Plant Extracts

Acacia gourmaensis A. Chev (bark) and *Eclipta alba* (L.) Hassk. (whole plant) were collected in 2006 at Kamboinsé (Burkina Faso). Collected materials were air-dried and then reduced into powder using a mortar. The powders were sieved and only particles smaller than 2.0 mm were used. In each case, 25 g of powder were mixed with 100 mL of distilled water and the mixture was incubated at 25-30°C for 20 h. Extracts were subsequently filtered with cheesecloth and centrifuged at 3000 rpm for 5 min to remove plant debris. Finally, supernatants were filtered with a coffee filter before use.

Two commercial chemicals (Benlate T20 and Apron star 42 WS) were used as positive controls for sorghum and pearl millet, respectively. Benlate T20 was composed of 20% Benomyl and 20% Thirame while Apron star 42 WS contained 20% Thiamethoxam, 20% Metalaxyl-M and 20% Difenoconazole.

Sorghum and Pearl Millet Seed Samples

Sorghum cultivar Sariaso 11 and pearl millet cv. IKMP 5 were used in this study. Seed samples of these two cultivars were collected from farmers' saved-seed lots in 2006. Preliminary tests indicated that collected seeds were infected by the following fungi: *Fusarium moniliforme* Sheldon (syn *F. thapsinum* Klittich, Leslie, Nelson et Marasas sp. nov.), *Curvularia lunata* (Wakk.) Boedijn, *Phoma sorghina* (Sacc.) Boerema, Dorenbosch and Van Kesteren, *Colletotrichum graminicola* (Ces.) Wilson (syn *C. sublineolum* P. Henn) and *Exserohilum rostratum* (Drechsler) Leonard and Suggs.

Seed Treatment

Naturally-infected seeds of sorghum and pearl millet were first immersed for 20 h in plant extracts at 25-30°C in the dark. Then they were air-dried for another period of 20 h. Control treatments included non-treated seeds, seeds immersed in distilled water and seeds treated with commercial chemicals i.e., Benlate T20 (5 g kg^{-1} of seed) and Apron star (2.5 g kg^{-1} of seed).

Testing the Effect of Aqueous Extracts on Seed Health

For each crop species, 200 seeds per treatment were tested for seed health using the standard blotter method reported by Mathur and Kongsdal (2003). Seeds were sown on moistened blotters in Petri dishes (25 seeds per Petri dish) and incubated for 7 days at 20-25°C under alternating cycles of 12 h of near-ultraviolet light and 12 h of darkness. Incubated seeds were examined individually and the growing fungi were recorded.

Testing the Effect of Aqueous Extracts on Seedling Emergence, Seedling Mortality and Plant Growth

For each crop species, 200 seeds per treatment were sown in plastic pots containing sterilized soil (25 seeds per pot). The soil was saturated with water before sowing. The pots were placed at 25-30°C under alternating cycles of 12 h daylight and 12 h of dark. The pots were watered regularly to maintain the moisture. The number of emerged seedlings as well as that of subsequently dead seedlings was recorded 15 Days After Sowing (DAS). To evaluate plant growth, seedling fresh weight was recorded at 30 DAS.

Field Condition Tests

The effect of the aqueous extracts on seedling emergence, seedling mortality, plant vigour and grain yield was evaluated by sowing the treated seeds and growing plants under field conditions. In total, three trials for sorghum and four trials for pearl millet were conducted in farmers' fields. All trials were implemented using a randomised complete bloc design with four replicates. In each plot, mineral fertilizer consisting of Nitrogen-Phosphate-Potassium (NPK 14-23-14) was applied at 100 kg ha^{-1} at sowing. Urea (50 kg ha^{-1}) was also applied 30 days post-emergence. In any case, seeds were sown in 6 m-long rows (6 rows par plot) with distances of 0.80 m between rows and 0.40 m between holes in the same row. Data were recorded from the four central rows of each plot. These included:

- The number of holes with germinated seeds, 15 DAS
- The number of holes containing one or more dead seedlings, 15 DAS
- Plant vigour estimated 21 DAS, with vigour ratings based on a 1-10 scale, where 1 = poor vigour and 10 = excellent vigour (Csinos *et al*., 2000)
- Grain yield, expressed in kg ha^{-1}

Statistical Analyses

The data recorded were analysed by ANOVA procedures using Statistical Analysis System, version 8. Significant differences between treatment means were determined using Least Significant Difference (LSD) (Gomez and Gomez, 1984).

RESULTS

Laboratory Tests

Laboratory tests were aimed at assessing the effects of seed treatment with aqueous plant extracts on the control of seed contamination by fungi, the emergence and mortality of seedlings and the seedling growth.

Treatments of Sorghum Seeds

Plant extracts and the commercial chemical Benlate T20 had significant effects on controlling seed infection by *P. sorghina* (Table 1). Only distilled water gave results which were similar to those from non-treated seeds. Infections by *P. sorghina* were reduced by 27.0, 72.0 and 64.0%, respectively with extracts of *A. gourmaensis* and *E. alba* and the chemical control Benlate T20. *A. gourmaensis* and Benlate T20 also lowered *C. graminicola* incidence on seeds by 68.5 and 75.1%, respectively. Among the treatments, Benlate T20 was the only one effective against *C. lunata* (93% reduction of incidence). None of the treatments had significant effect on *F. moniliforme*. Compared to non-treated seeds, seedling emergence and mortality were reduced by the chemical control only. Treatment of seeds with Benlate T20 lowered seedling emergence by 15.8% but preserved all the emerged seedlings from death. Seed treatment with *A. gourmaensis* and *E. alba* stimulated the weight of the seedlings. Seedling growth was most stimulated by extract of *A. gourmaensis* which led to the heaviest seedling weight.

Treatments of Pearl Millet Seeds

Extracts of *A. gourmaensis* and *E. alba* effectively controlled seed infection by all fungi, except *E. rostratum*. Compared to non-treated seeds, seed infections were reduced at rates ranging between 69 to 86.4% and 52.1 to 72.0%, respectively (Table 2). The chemical Apron star was effective in controlling seed infection by *F. moniliforme* and *P. sorghina* only. None of the treatments showed significant effects on seedling emergence and mortality. However, as observed in sorghum, the treatment of seeds with *A. gourmaensis* and *E. alba* increased seedling weight, the most biomass promoting treatment being that with *A. gourmaensis*.

Field Trials

Field trials were conducted to assess the effect of seed treatment with plant extracts and chemical fungicides on emergence and mortality of seedlings, plant vigour and grain yield.

Table 1: Effect of treatment of sorghum seeds with aqueous plant extracts on incidence of four seed-borne fungi, emergence and mortality of seedlings and plant biomass[a]

Treatments	Percent of infected seeds by fungi				Emerged seedlings (%)	Dead seedlings (%)	Seedling fresh weight (g)
	C. graminicola	*C. lunata*	*F. moniliforme*	*P. sorghina*			
A. gourmaensis	6.7bc	7.3a	13.3a	48.7b	79.3ab	17.3a	22.7a
	(68.5)[b]	(27.0)	(16.9)	(27.0)	(10.6)	(45.5)	(- 24.0)
E. alba	15.3ab	10.0a	13.3a	18.7c	84.7ab	16.6a	22.0ab
	(28.2)	(0.0)	(16.9)	(72.0)	(4.5)	(47.8)	(- 20.2)
Chemical control	5.3c	0.7b	12.7a	24.0c	74.7b	0.0b	-[c]
(Benlate T20)	(75.1)	(93.0)	(20.6)	(64.0)	(15.8)	(100.0)	
Distilled water	12.0bc	6.7ab	14.0a	72.7a	80.7ab	29.8a	-
	(43.7)	(33.0)	(12.5)	(-9.0)	9.0)	(1.0)	
Non-treated seeds	21.3a	10.0a	16.0a	66.7a	88.7a	30.1a	18.3b
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)

[a]Tested fungi were *Colletotrichum graminicola*, *Curvularia lunata*, *Fusarium moniliforme* and *Phoma sorghina*; Means in columns followed by the same letter(s) are not significantly different (p = 0.05) according to LSD test; [b] Percentage of reduction compared to non-treated seeds; [c] - Non evaluated

Table 2: Effect of treatment of pearl millet seeds with aqueous plant extracts on incidence of four seed-borne fungi, emergence and mortality of seedlings and plant biomass[a]

Treatments	Percent of infected seeds by fungi: *C. lunata*	*E. rostratum*	*F. moniliforme*	*P. sorghina*	Emerged seedlings (%)	Dead seedlings (%)	Seedling fresh weight (g)
A. gourmaensis	13.0b	2.5a	5.0c	9.0c	76.0a	6.7a	28.4a
	(69.0)[b]	(70.6)	(86.0)	(86.4)	(1.9)	(-272.2)	(-39.2)
E. alba	18.5b	11.0a	17.0b	18.5b	67.0a	2.2a	21.5ab
	(56.0)	(-29.4)	(52.1)	(72.0)	(13.5)	(-22.2)	(-5.4)
Chemical control	36.5a	4.0a	21.5b	11.5bc	75.0a	4.4a	-[c]
(Apron star 42)	(13.1)	(52.9)	(39.4)	(82.6)	(3.2)	(-144.4)	
Distilled water	23.0b	7.5a	9.0c	67.0a	61.0a	3.4a	-
	(45.2)	(11.8)	(74.6)	(-1.5)	(21.3)	(-88.9)	
Non-treated seeds	42.0a	8.5a	35.5a	66.0a	77.5a	1.8a	20.4b
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)

Tested fungi were *Curvularia lunata*, *Exserohilum rostratum*, *Fusarium moniliforme* and *Phoma sorghina*; Means in columns followed by the same letter(s) are not significantly different (p = 0.05) according to LSD test; [b]Percentage of reduction compared to non-treated seeds; [c]- Non evaluated

Table 3: Effect of treatment of sorghum seeds with aqueous plant extracts on emergence and mortality of seedlings, plant vigour and grain yield

Treatments	Emerged seedlings (%)	Dead seedlings (%)	Plant vigour rating[a]	Grain yield (kg ha^{-1})
A. gourmaensis	80.2a	0.6a	7.1a	1669.6b
E. alba	78.5a	1.4a	8.3a	2488.4a
Chemical control	70.6ab	1.1a	7.8a	1999.3ab
Non-treated seeds	66.4b	0.6a	4.3b	1461.3b

[a]Plant vigour ratings based on a 1-10 scale, where: 1 = poor and 10 = excellent; Means in columns followed by the same letter(s) are not significantly different (p = 0.05) according to LSD test

Table 4: Effect of treatment of pearl millet seeds with aqueous plant extracts on emergence and mortality of seedlings, plant vigour and grain yield

Treatments	Emerged seedlings (%)	Dead seedlings (%)	Plant vigour rating[a]	Grain yield (kg ha^{-1})
A. gourmaensis	84.2ab	4.1ab	7.4b	1490.9ab
E. alba	80.4b	9.1a	8.1ab	1631.9a
Chemical control	88.6a	1.6b	8.4a	1642.8a
Non-treated seeds	81.5b	6.8ab	6.3c	1299.9b

[a]Plant vigour ratings based on a 1-10 scale, where: 1 = poor and 10 = excellent; Means in columns followed by the same letter(s) are not significantly different (p = 0.05) according to LSD test

Effects of Treatments of Sorghum Seeds

Seed treatment with plant extract and chemical Benlate T20 had significant effects on emergence of seedlings (F = 2.85; p = 0.04). Treatments of the seeds with extracts of *A. gourmaensis* and *E. alba* resulted in 80.2 and 70.6% of seedling emergence, which were significantly higher than the proportion of seedlings emerged from non-treated seeds (66.4%) (Table 3). The chemical Benlate T20 had no marked effect on seedling emergence. In all treatments, seedling mortality was low (0.6 to 1.6%) and no effect of the treatments were noticed (F = 0.79; p = 0.56). By contrast, plant vigour ratings were significantly higher in seedlings emerged from all treated seeds (7.1 to 8.3) than in the case of non-treated control seeds (F = 14.17; p<0.01). Overall, plant yield was also significantly affected by treatments of the seeds (F = 2.61; p<0.05). However, only treatments with extracts from *E. alba* induced a clear increase in grain yield. With these treatments, average yield reached 2.5 t ha^{-1} while in all other cases yields did not exceed 2 t ha^{-1}.

Effects of Treatments of Pearl Millet Seeds

As indicated in Table 4, treatments of pearl millet seeds with plants extracts and commercial chemical Apron star resulted in significant effects on emergence (F = 2.86; p = 0.04) of seedlings, plant vigour (F = 8.42; p<0.01) and grain yield (F = 2.01; p<0.05). Proportions of emerged seedlings from treated seeds of pearl millet varied between 80.4 to 88.6% and only the commercial chemical control performed significantly better than the non-treated seeds. Effects of seed treatments on seedling mortality were less apparent (F = 2.92; p = 0.06) and the highest mortality (9.1%) was induced by treatments with plant extract from *E. alba*. More clear-cut seed treatment effects were observed on plant vigour. All seed-treating products significantly induced higher plant vigour compared to the non-treated seeds. Moreover, the commercial chemical Apron star performed better than the two plants extracts. As for grain production, only seed treatments with plant extract from *E. alba* and Apron star gave yields which were significantly higher than that of plants from non-treated seeds.

DISCUSSION

Low seed quality is a serious problem in crop production, especially in most developing countries. For instance, the use of infected seeds without any treatment against infecting pathogens is often responsible for the decrease in seedling emergence (Hofs *et al*., 2004). This effect may even continue to act on plant growth, therefore affecting plant vigour and yield. Fungi such as *Fusarium moniliforme*, *Phoma sorghina*, *Curvularia lunata*, *Colletotrichum graminicola* and *Exserohilum rostratum* were frequently isolated from many sorghum and pearl millet seed samples collected in different locations of Burkina Faso (Zida *et al*., 2008). In the present study, aqueous extracts of *A. gourmaensis* and *E. alba* showed effectiveness in controlling several sorghum and pearl millet seed-borne fungi. The fungus *P. sorghina* was controlled by both plant extracts in sorghum seeds. However, plant extract from *A. gourmaensis* had more fungicidal properties because it was the only one capable to control *C. graminicola*. The development of *C. lunata* was efficiently inhibited by the chemical Benlate T20 only. This result indicated that Benlate T20 was more effective than the two plant extracts in controlling sorghum seed-borne fungi.

By contrast, plant extracts were more efficient than the chemical Apron star in the control of fungi in pearl millet seeds. Treatments of the seeds with Apron star had no significant effect in inhibiting *C. lunata*, in the contrary of plant extracts. *F. moniliforme* was efficiently controlled by plant extracts in pearl millet seeds but not in those of sorghum. This result suggests the occurrence of variants of the fungus with different levels of resistance to fungicides. Depending on the plant species from which seeds *F. moniliforme* was isolated, Kini *et al*. (2002) recently found specific genetic variants of the fungus in the country.

Both *A. gourmaensis* and *E. alba* extracts promoted plant growth. Seedling weight after germination was enhanced upon treatment of the seeds by either extract, regardless of the crop species. Similar increase in seedling fresh weight after treatment of rice seeds with fungal and bacterial bio-control agents have been reported (Mishra and Sinha, 2000). Extracts from *A. gourmaensis* were most efficient in promoting plant growth. The effect of both plant extracts on plant growth is possibly favoured by their upstream effects on controlling seed-borne fungi. However, other factors may be involved in the process, as in some cases plant growth was promoted while extracts were not efficient in controlling seed pathogens.

Field tests confirmed the beneficial effects of treating both sorghum and pearl millet seeds for the control of seed-borne fungi. On the one hand, emergence and vigour of seedlings were generally increased in both crops when the seeds were treated either with plant extracts or chemical products. On the other hand, treatments of the seeds did not affect the mortality of emerged seedlings significantly. Consequently, grain yields were increased due to treatments of the seeds. Treatments of sorghum or pearl millet seeds with plant extracts from *E. alba* led to the most increase in yield. Altogether, plant extracts showed an overall good performance in promoting seed health against infections by fungi and enhancing grain yields.

The fungicidal effects of *E. alba* extracts reported here are consistent with results obtained by Kaushal *et al*. (2002) and Venkatesan and Ravi (2004). These authors have found high inhibitory effects of extracts of *E. alba* against fungi such as *Macrophomina phaseolina*, *Candida tropicalis*, *Rhodotorula glutini* and *Candida albicans*. The antifungal activity of aqueous bark extracts from *Acacia gourmaensis* are reported for the first time. The inhibitory effects of both *A. gourmaensis* and *E. alba* on fungi can be attributed to active chemical components within the plants. However, more investigation is needed to identify the chemical nature of these components.

Overall, results obtained in this study underlined the possible used of aqueous extracts of *E. alba* and *A. gourmaensis* by smallholder farmers to control several seed-borne fungi. These include fungi infecting pearl millet seeds (*Phoma sorghina*, *Curvularia lunata* and *Fusarium moniliforme*) and those infecting sorghum seeds (*P. sorghina* and *Colletotrichum graminicola*). Extract from both *E. alba* and *A. gourmaensis* may be used as alternatives to commonly used chemicals for seed treatment against fungi. Both plant species are widely distributed in sub-Saharan Africa. However, further investigation is needed in order to determine optimal concentrations of the extracts. Investigation on the mammal toxicity of the extracts is also required. Testing the effect of extracts from organs other than bark, especially for *A. gourmaensis* may also be useful for better preservation of this species.

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