Seed treatment with a binary pesticide and aqueous extract of *Eclipta alba* (L.) Hassk. for improving sorghum yield in Burkina Faso

Elisabeth P. Zida[1]*, Ole Søgaard Lund[2], and James B. Néya[1]

[1]*Institute of Environment and Agricultural Research, P.O. Box 7192, Burkina Faso;* [2]*University of Copenhagen, Dep. Agriculture and Ecology, Højbakkegaard Alle 19, 2630 Taastrup, Denmark*

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Abstract

Seed health testing and field trials were carried out in Burkina Faso to evaluate the effects of seed treatment with aqueous extracts of *Eclipta alba* and a binary pesticide Calthio C 50 WS (thiram 25%, chlorpyrifos ethyl 25%) on seed-borne fungi, seedling emergence, and yield of sorghum. The treatments included soaking seeds with *E. alba* extract (6.25 to 25% concentrations), soaking seeds in water, dusting seeds with pesticide (4 g kg^{-1} of seeds), and no treatment. Seed samples with moderate to high infection levels of *Curvularia* Boedijn., *Fusarium* Link: Fr., and *Phoma* Sacc. were used for testing. *E. alba* extract and Calthio C caused significant inhibition of *Leptosphaeria sacchari* Breda de Haan (syn. *Phoma sorghina* Sacc.) *in vitro*. In field trials, a stimulatory effect on seedling emergence and yield increases of 7 to 38% were observed for *E. alba* and the pesticide treatments, as compared to no treatment. Yield increases were significant for plant extract and pesticide in two out of three trials. No consistent effect on yield was observed for water treatment. Our findings represent the first large-scale field testing of *E. alba* for seed treatment of sorghum and the first direct comparison with a thiram-based pesticide and seed priming.

Keywords: Plant extract, Antifungal activity, Seed-borne fungi, Thiram, Seedling emergence.

Introduction

In Nigeria, Ghana and Burkina Faso, *Fusarium*, *Curvularia,* and *Phoma* are among the most common sorghum seed mycoflora (Zida et al., 2008a). These pathogens are transmitted and disseminated by seeds. They affect seed germination and cause diseases in seedlings and plants. Grain mould is one of the major sorghum diseases caused by *Fusarium*, *Curvularia,* and *Phoma*. The seed-borne fungi (e.g., *Fusarium* spp.) produce mycotoxins that pose health risks to humans and animals. In farm-saved seeds from Burkina Faso, *Leptosphaeria sacchari* Breda de Haan (syn. *Phoma sorghina* Sacc.) infection often exceeded 30% and this fungus is one of several species having the ability to cause grain mould and seed rot in sorghum (Girish et al., 2004a).

The use of fungicides is an effective strategy to control seed-borne fungal diseases in crops. Treatment of sorghum seeds with both fungicide and insecticide is generally recommended (FAO, 2011). In Western Africa, a binary pesticide, Calthio C, containing the fungicide, thiram, and the insecticide, chlorpyrifos-ethyl, is widely available for seed treatment as dry powder. Seed treatment with thiram-based fungicides has been reported to improve germination and/or yield of sorghum (Girish et al., 2004b). But, the resource poor African sorghum farmers usually find it difficult to apply commercial pesticides. Hazardous effects on the environment and induction of genetic resistance due to repeated use of synthetic pesticides are other major problems associated with the use of such chemicals.

*Author for correspondences: Phone + 226 70 06 16 72; Email <zidap.elisabeth@yahoo.fr>.

In many instances, botanical extracts are reported as effective and are cheaper alternatives to chemicals in combating insect pests and fungal diseases in plants (Tegene and Pretorious, 2007; Zida et al., 2008a). Recently, a study in Burkina Faso reported that seed treatment of sorghum with an aqueous extract (25% concentration) of a local plant, *Eclipta* a*lba* (Asteraceae), markedly reduced seed infection by *Phoma* sp. (Zida et al., 2008b). In the same study, a positive impact on sorghum grain yield was observed by applying the plant extract for seed treatment in a small-scale field trial. The use of *E. alba*, a medicinal plant, in seed treatment represents a low risk activity compared to the use of synthetic pesticides; and this plant has the potential to provide a low-cost alternative to synthetic fungicides. The objective of this study was to evaluate the potential of applying *E. alba* extract or the chemical Calthio C to combat fungal infection in seeds of sorghum and to obtain a substantial yield increase in a large scale field study.

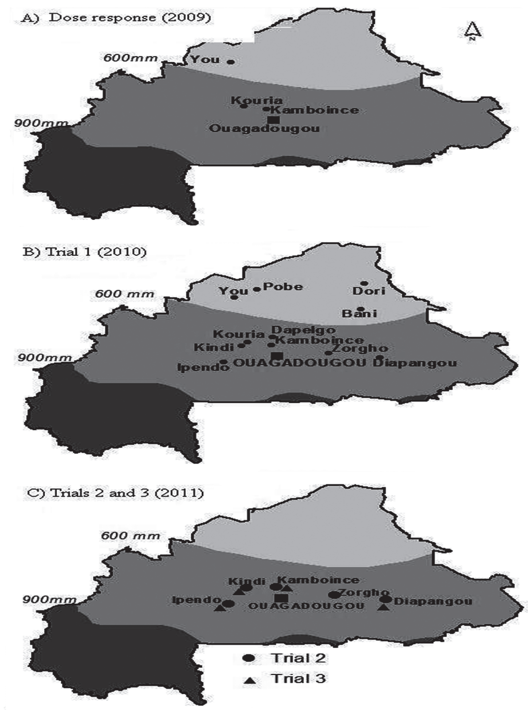

Figure 1. Distribution of field trial sites in Burkina Faso, 2009–2011

Materials and Methods

Bioassays were carried out in the Laboratory of Phytopathology at Kamboinse Research Centre (Ouagadougou) and field experiments in two agroecological zones in Central, Eastern, and Northern Burkina Faso, West Africa (Fig. 1). Naturally infected farm-saved seeds of sorghum infected by fungal pathogens (*Curvularia*, *Fusarium*, and *Phoma*) were used. Nine seed samples infected by *L. sacchari* were collected from northern, eastern, and central Burkina Faso in 2009 for testing dose response of seed treatment with *E. alba* extract. One large seed sample infected by *Phoma* (*L. sacchari)*, *Curvularia* spp., and *Fusarium* spp. was collected from a single farmer in Central Burkina Faso in 2010 and the same was done in 2011 for field trials. In 2011, five other seed samples were also collected from the central, northern, and eastern Burkina Faso and used in separate experiments.

Leaves and stems of *E. alba* were collected from wild plants growing in the humid sites near Ouagadougou, Burkina Faso. The material collected was air-dried, ground, and sieved with a mesh of 2 mm diameter. The powder was mixed with distilled water at the desired concentrations on a W/V basis and incubated at 25 to 30°C for 20 h. Although three concentrations (6.25%, 12.5%, and 25%) were tested in 2009, only the 12.5% and 10% concentrations were used in 2010. Aqueous extracts were obtained by filtering the mixtures with a piece of cloth (hand filtering).

In all experiments, seed treatment with *E. alba* extract consisted of soaking naturally infected seeds in the extract for 10 to 20 h. Seeds were soaked in the plant extract for 20 h for experiments carried out during 2009 and 2010 and for 10 h for the experiments conducted in 2011. The soaked seeds were dried for 20 h before use. Non-treated seeds were used as control. Seed treatment with Calthio C was carried out by dusting seeds by the application of 4 g pesticide powder per kilogram of seeds, according to manufacturer's recommendation. In order to separate any specific effects caused by the extract of *E. alba* from any general effect of water, a water soaking treatment was included in the large-scale trials carried out in 2010 and 2011, as control, by immersing seeds in distilled water and drying them in a similar manner as for the *E. alba* extract treated seeds.

Before initiation of the field trials, antifungal activity of *E. alba* extract at different concentrations (6.25%, 12.5%, and 25%) on seeds was tested, using the standard blotter method (Mathur and Kongsdal, 2003). Seeds treated with Calthio C and non-treated seeds were also tested. For each of the nine seed samples used and for each treatment, 200 seeds were germinated on moistened blotters in petri-dishes (25 seeds per petri-dish) and incubated for 7 days at 22°C under alternating cycles of 12 h near-ultra-violet light (Philips TLD 36W/BLB) and 12 h darkness. Incubated seeds were examined individually in stereo (Wild M5A, magnification up to 50x) and compound (Leitz Laborlux S, magnification up to 400x) microscopes and the number of seeds infected by *L. sacchari* was recorded.

Seeds treated with *E. alba* extract (6.25%, 12.5%, and 25%) and Calthio C and non-treated seeds were also tested for emergence and grain yield in a small-scale trial. Seeds were sown in six fields in central and northern Burkina Faso (Fig. 1A). Different seed treatments were tested as single plots in each field. In all fields, mineral fertiliser 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. Seeds were sown in 6 m long rows (20 rows per plot) at distances of 0.80 m between rows and 0.40 m between holes in the same row. Four to six seeds were sown per hole and at 15 days after sowing, the number of emerged seedlings per hole was thinned to a maximum of four. At harvest, grain weight was recorded from the 18 middle rows of each plot, two weeks after drying.

Based on the results obtained from the dose response experiments (2009), *E. alba* extract concentration close to 10% (12.5% and 10%) were selected for seed treatment in 2010 and 2011. Four seed treatments (soaking of seeds in pure water, soaking of seeds in *E. alba* extract, dusting of seeds with Calthio C and no treatment of seeds) were compared. Antifungal effects of seed treatment were evaluated by using the blotter test method, as described above. The percentages of seeds infected by specified species (*Phoma* sp., *Fusarium* spp. and *Curvularia* spp.) were determined.

Seedling emergence and grain yield were evaluated in three large scale trials in 2010 and 2011, using a randomised complete block design with three replicates of each of the four seed treatments. In Trial 1 conducted during 2010, 23 farmers' fields were included (Fig. 1B) and in Trial 2 during 2011, 17 farmers' fields were included (Fig. 1C). In Trial 3 (2011), a total of 13 fields were included (Fig. 1C). Seeds were sown in five 5 m long rows per plot. Cultivation practices as described for the dose response experiment on crop yield conducted during 2009 were practiced. Data were recorded from all the five rows per plot and included emergence (percentage of seed holes with emerging plants 2 to 3 weeks after sowing) and yield. Data on inhibition of *L. sacchari*, emergence and yield were analyzed with software PAST version 2.1 for calculating intervals of 95% confidence and *p* values of significant differences by two sample comparison in students *t*-test assuming normal distribution of data. For the field trials 1 to 3, the average for each field of all three replicates was used as a single value. Relative yields ($\%^R$) were calculated as grain yield for each treatment as percentage of the average of all four treatments within the same field. One way analysis of variance was performed on the data on distribution of fungi on seeds and Least Significant Difference (LSD) test was used for mean comparison, using SAS software (Statistical Analysis System, version 8 for Windows, SAS Institute Inc.).

Results and Discussion

In non-treated seeds, the average infection level was 40% for *L. sacchari* and a significant and dose-dependent inhibition of this fungus was observed for the *E. alba* extract (Fig. 2A). For the 12.5% *E. alba* extract, fungal inhibition close to 80% was observed ($p < 0.001$). However, stronger fungal inhibition (97%) was observed for treatment with Calthio C ($p < 0.001$). With respect to crop yield, average yields obtained varied from 749 to 1082 kg ha^{-1} (Fig. 2B). Overall increases in yield ranged from 17% (*E. alba* high dose) to 44% (Calthio C).

The efficacy of seed treatment with an aqueous extract of *E. alba* and a binary pesticide Calthio C in controlling seed-borne fungi of sorghum and in enhancing yield was demonstrated in the present study. A manifest dose response of *E. alba* extract with regard to its antifungal activity towards seed-borne inoculum of *L. sacchari* was also demonstrated. The highest concentration of *E. alba* extract (25%) appeared to have an unfavorable effect on yield compared to the lower doses, but the low number of determinations (N = 6) did not allow a robust statistical testing. However, the results obtained for both antifungal effect and yield prompted us to test concentrations of *E. alba* extract close to 10% (W/V) in the subsequent field trials.

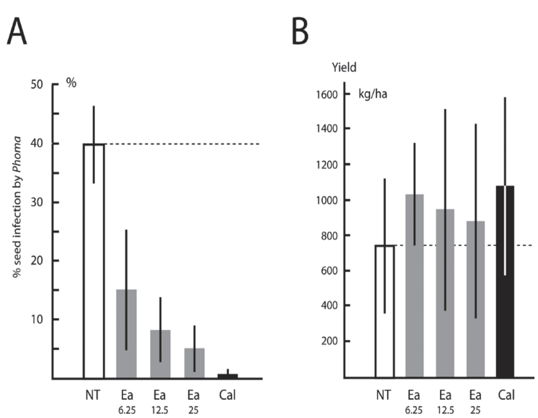

Figure 2. Effects of seed treatment with three concentrations of *E. alba* extract (Ea: 6.25% ; 12.5%; 25%) and a soaking time of 20 h tested against non-treated seeds (NT) and Calthio C (Cal). **A)** Antifungal effect: percentage of seeds infected with the *Phoma* species, *L. sacchari*, were enumerated in blotter test on nine samples of farm saved seeds incubated for seven days after treatment. **B)** Grain yield of Sorghum obtained in six farmers fields in Central and Northern Burkina Faso (year 2009) four months after sowing seeds treated as shown. Small bars indicate 95% confidence interval of mean.

From blotter test results, it appeared that application of Calthio C was inhibitory to *Phoma* and *Curvularia* (Table 1). Soaking seeds in *E. alba* extract for 20 h caused inhibition of *Phoma,* as previously observed in the dose response experiments (Fig. 2A). However, reduction of soaking time to 10 h almost eliminated the inhibition of both *Phoma* and *Curvularia* (Table 1). In all trials, crop emergence rates were higher on *E. alba* extract treated seed (77 to 83%) and Calthio C (82 to 97%) than seeds treated with pure water or no treatment (71 to 77%; Fig. 3). Thiram-based fungicides have been reported to increase germination in sorghum seeds and yield increases up to 30% were reported (Girish

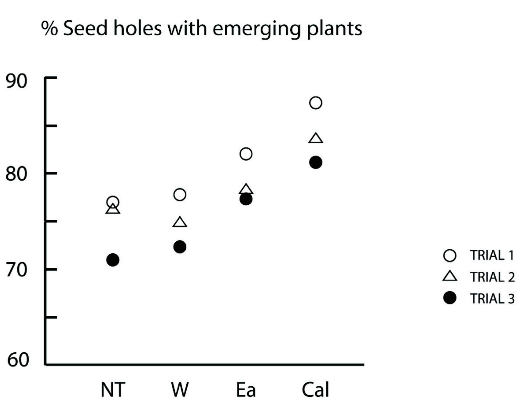

Figure 3. Seed treatments and crop emergence in field trials in Burkina Faso. The average percent of seed holes with emerging plants three weeks after sowing is shown for non-treated seeds (NT), water (W), *E. alba* extract (Ea) and Calthio C (Cal).

Table 1. Effect of seed treatment on mycoflora on seeds.

Genus	Treatment	Percentage of seeds infected relative to NT (NT=100) (Absolute values of NT shown in Table 2)			
		Trial 1 12.5%–20 h	Trial 2 12.5%–10 h	Trial 3* 10%–10 h	Mean
Phoma	NT	100	100	100	100
	W	145	109	117	124
	Ea	9	83	81	58
	Cal	0	34	11	15
Curvularia	NT	100	100	100	100
	W	38	106	83	75
	Ea	39	88	72	66
	Cal	6	40	23	23
Fusarium	NT	100	100	100	100
	W	207	95	82	128
	Ea	314	88	113	172
	Cal	57	38	65	53

* average of five seed samples; NT = non-treated seeds; W = water soaking; Ea: *Eclipta alba;* Cal = Calthio C.

et al., 2004b). However, this is the first report on an extension of these findings to a large-scale field testing, comparing a specific control (water treatment) and a relevant pesticide containing the antifungal compound, thiram.

With respect to the effect on crop yield, the lowest yields were obtained for non-treated seeds and those treated with distilled water and the highest yields for seeds treated with *E. alba* extract and Calthio C (Table 2; Fig. 4). Seed treatments with *E. alba* extract and Calthio C significantly ($p < 0.01$) increased yield compared to non-treated seeds. Normalized yields (relative to non-treated seeds) were 117% for *E. alba* and 125% for Calthio C (Table 2). Significant differences were also found between *E. alba* and water and between Calthio C and water. Antifungal effect of plants and positive effects on yield and/or germination following application of botanical extracts have been demonstrated earlier in sorghum (Tegene and Pretorious, 2007; Raghavendra et al., 2007; Wulff et al., 2012).

Consistent and significant improvement of the emergence rate and yield was observed for both pesticide and plant extract, when tested in two different growing seasons. With regard to the antifungal effects and yield increases, Calthio C appeared to be the most promising inhibitor of *Curvularia* and also the most efficient promoter of yield, particularly when seeds with a high content of *Curvularia* were tested (Table 1). However, it could be premature to conclude anything regarding the mechanisms involved in yield increases based on these observations. Nonetheless, it may indicate the relevance of a more extensive comparison of *E. alba* extract and Calthio C treatment of seeds with different infection levels and profile. At present, the effect of *E. alba* extract on yield is evident but the biological mechanism involved appears rather uncertain and may depend on the concentration and soaking time.

The use of an aqueous plant extract implies exposure of seeds to water and several previous studies have reported significant yield increases (> 20%) following seed priming, using a short exposure time (8–10 h). In the present study, an increase of yield (+11% normalized yield) was observed in one of the two trials employing the short soaking time (10

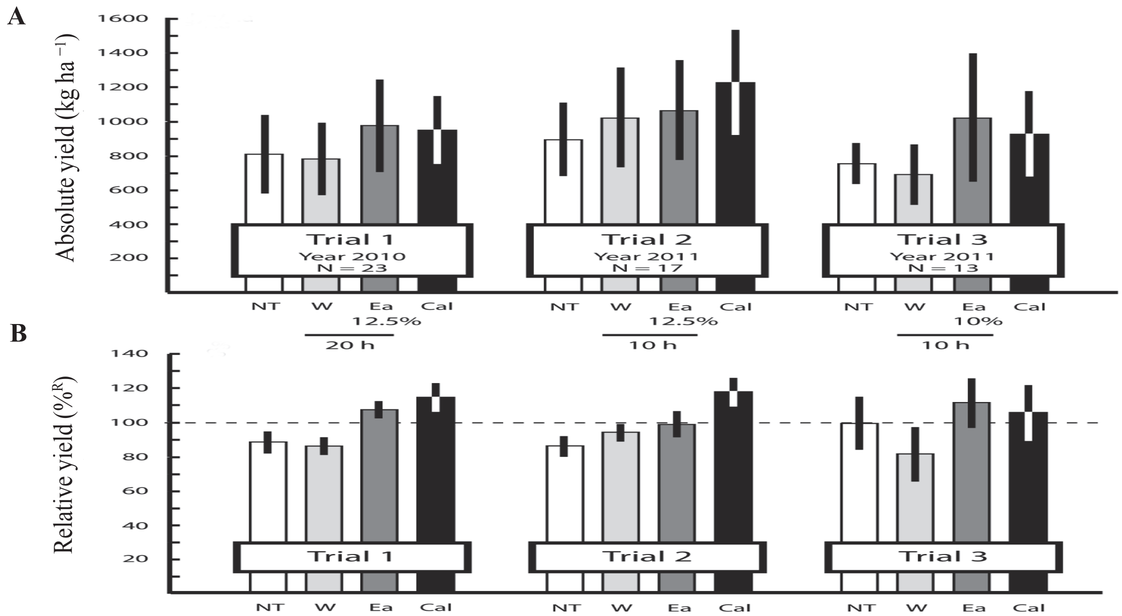

Figure 4. Effect of four seed treatments on grain yield. In all trials non-treated seeds (NT) were compared to seeds treated with pure water (W), *E. alba* extract (Ea) and Calthio C (Cal). **A)** Absolute yield (kg/ha) calculated for each treatment. The number of fields included (N), the year of testing, soaking time (hours) and concentration of *E. alba* extract (W/V %) applied are shown for each trial. **B)** Relative yield ($\%^{R}$) calculated as the average for each treatment relative to the average for all 4 treatments within each field. Small bars indicate 95% confidence interval of mean.

Table 2. Levels of infection of seed samples and normalized yield of sorghum recorded in three large scale trials in Burkina Faso.

Trial	Fields (N), year	Experimental conditions	% Fungal infection (non-treated seeds)			Normalized Yield ($\%^{N}$)			
			Phoma	*Curvularia*	*Fusarium*	NT	W	Ea	Cal
1	N=23, 2010	Ea 12.5% 20h soaking	17	42	7	100^{a}	98^{a}	122^{b}	130^{b}
2	N=17, 2011	Ea 12.5% 10h soaking	25	38	37	100^{a}	111^{ab}	116^{b}	138^{c}
3*	N=13, 2011	Ea 10% 10h soaking	38	8	24	100^{ab}	87^{b}	113^{a}	107^{a}
Mean	26	29	23	100	99	117	125		

Ea= *Eclipta alba;* NT=non-treated seeds, W=soaking seeds in water, Cal=dusting seeds with pesticide (4 g kg^{-1} of seeds).
*For this trial figures of fungal infection are the average of five seed samples used.
Means on the same row followed by the same letter are not significantly different, using *t*-test at 5% level.

h), whereas a marked decrease of yield (–13% normalized yield) was observed in the other. Differences between the seed material used in different trials seems a plausible explanation of this inconsistency. Nevertheless, the effect of plant extract on yield and emergence cannot be explained as a simple exposure of seeds to water (seed priming) and a dose dependent antifungal activity was demonstrated against *L. sacchari*, commonly found on seeds of sorghum in Burkina Faso.

In conclusion, an important finding in the present study is the demonstration that a simple aqueous extract of a local plant, *E. alba*, is capable of increasing sorghum yields when tested on a larger number of fields and when applied to farm-saved seeds carrying a natural inoculum of mycoflora. Although several questions are still unanswered, the study demonstrates the potential of antifungal seed treatment in Sahel.

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