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# Increased feasibility of treating sorghum seeds with *Eclipta alba* extract by lowering concentration of plant extract and soaking time of seeds

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***ABSTRACT:*** *Previous field experiments in Burkina Faso have demonstrated the efficacy of an aqueous extract of the plant, Eclipta alba in improving crop emergence and yield of sorghum by soaking of seeds for 10-20 hours in 10-25% (w/v) extract. When the effect of seed treatment was tested in vitro using seedling vigour as the response parameter, results indicated that lowering of the concentration of plant extract (from 10% to 2%) and shortening of the soaking time (from 10 h to 6 h) resulted in a similar response as the higher dose and longer soaking time.In a series of field experiments a yield increase of more than 150 kg/ha was obtained either by applying a low concentration of plant extract (2.5% w/v) or a short soaking time (6 h).The low dose and reduced soaking time was further combined in a field trial and resulted in a 180 kg/ha yield increase compared to non-treated seeds. This corresponded to a relative yield increase of 16-27% at field level($p< 0.0042$). Also crop emergence was increased significantly (7%) by treatment with low dose and shortened soaking time ($p< 0.005$). Experiments in vitro supported the findings and demonstrated a protective activity of the plant extract: the frequency of seedlings with symptoms of seed-borne fungal infection in roots was reduced more than three-fold in plants from treated seeds ($p<0.001$). Naturally infected seed material was used in all experiments and the findings are therefore directly applicable and relevant for farmers in Burkina Faso, where E. alba is abundant.*

## INTRODUCTION

Sorghum is an important cereal crop cultivated in many hot and dry areas due to itsheat and drought tolerance (Rooney 1996; Dicko *et al*. 2006). Although the crop is very robust to abiotic stresses it can be attacked by a plethora of pests and diseases (Teetes and Pendleton 2000, Frederiksen 2000). For this reason, seed treatment of sorghum using a binary combination of fungicide and insecticide is recommended (Tarr 1954, FAO 2014). In Burkina Faso, we recently reported a yield increase of 25% by application of the binary pesticide, Calthio C, in sorghum seed treatment (Zida 2012). However, in many developing countries seeds of sorghum are usually sown directly by hands without any protection of human skin. Therefore development of ecofriendly methods for seed treatment to avoid the use of synthetic chemicals toxic to humans is in demand.

In Burkina Faso, two previous studies have identified an aqueous extract of the medicinal and locally abundant plant, *Eclipta alba*, as a promising agent for seed treatment of sorghum. Initially, a small scale study of four field tests indicated a positive effect on both yield and emergence (Zida *et al*. 2008a). Subsequently a two-year trial including more than 25 different trial sites from different sorghum producing areas of Burkina Faso demonstrated on average 17% yield increase by the use of *E. alba* extract for seed treatment (Zida *et al*. 2012). In the latest study, the specificity of the effect was demonstrated by comparison to seed treatment with pure water (seed hydropriming)and to non-treated seeds. Both studies provided evidence, that the seed treatment could

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inhibit the fungus *Epicoccum sorghinum* (previously *Phoma sorghina*) on seeds. *E. sorghinum* is commonly found on seeds of sorghum in Burkina Faso (Zida *et al*. 2008b), but the pathogenicity of the fungus is not well documented. Also for two species of *Fusarium*: *F. oxysporum* and *F. glutinans*, dose-dependent inhibition by an aqueous exctract from leaves of *E. alba* (collected in India) have been reported (Saraswathy and Kumaran 2012) and activity against opportunistic fungal pathogens of humans, *Candida albicans* and *Aspergillus nige*r, has been demonstrated as well (Peraman *et al*., 2011, Borkataky *et al*., 2013).

In the previous field studies testing *E. alba* extract for seed treatment of sorghum, a relatively large amount of plant material: 10-25% w/v of whole plant dry matter was extracted and seeds were soaked for 10 hours or more in the extract. The relatively large amount of plant material applied is demanding in terms of the time needed to collect it and the swelling of hydrated plant material takes up a large volume during extraction and thereby reduces the free volume of the aqueous phase needed for soaking of seeds. In addition, the long soaking time of seeds (>10 hours) represents a risk factor with respect to seed quality: seed becomes more susceptible to mechanical stress during handling and sowing (Harris 1996) and emergence may decrease (Kaurivi and Shilulu 2007). For these reasons the aim of the present study was to test if reduction of the amount of plant material needed for extraction and reduction of the time needed for soaking of seeds could be applied and still retaining a positive effect on yield and emergence. Due to the large variation in yield obtained for sorghum in Burkina Faso (150-3500 kg/ha) the average yields of all trials were calculated both in absolute numbers (kg/ha) and as relative values compared to the average of all treatments within each individual field (experimental set of plots) as in Zida *et al*. 2012. Studies *in vitro* were conducted in parallel to test short time effects on vigour, growth and health of seedlings germinated under controlled conditions.

## MATERIALS AND METHODS

### Botanical extract and pesticides

*Botanical extract*: Whole plants of *E. alba* (L.) Hassk. were collected by uprooting (pulling by hand) of wild plants growing in fields and humid sites near Ouagadougou, Burkina Faso (used in field experiments and experiments in growth chamber) or near Mysore, India (used for *in vitro* vigour test). Soil particles on roots were removed by rinsing in tap water. Material collected was air-dried and reduced into powder by grinding and then sieved with a mesh of 2 mm diameter. Plant powder was stored in sealed plastic bags in darkness at room temperature for up to one year. Before application to seeds, plant powder was extracted by mixing with distilled water at appropriate concentrations (1.25%-12.5% w/v) and incubated for passive extraction at 25-30°C for 20 hours. The aqueous extract was filtered through a piece of cloth or roll bandage to remove large particular matter.

*Pesticide:* The binary pesticide, Calthio C, containing both the fungicide, thiram (25%), and the insecticide, chlorpyrifos-ethyl (25%) was obtained as dry powder from company Saphyto (Bobo-Dioulasso, Burkina Faso).A systemic fungicide Bavistin (Carbendazim 50% WP, BASF, India Ltd.) was obtained as dry powder from pesticide dealers in Mysore, India.

### Seed sampling and treatment

A total of 10 independent seed samples were used in the study: 7 samples were purchased from local farmers in Central and Northern Burkina Faso (Villages: Diapangou, Dapelgo, Kindi, Ipendo, Kouria and You), 2 samples (Cultivar Kapelga) were obtained from Research Organisation INERA, Burkina Faso and one sample ('Gundlupet local') was purchased from the seed market, Mysore, Karnataka, India. Seeds harvested for field trials were in all cases used in the subsequent growing season (no storage for more than 9 months). *E. alba* extract was applied by soaking of seeds for 1-24 hours in different concentrations of *E. alba* extract (1.25%-12.5%) as indicated for each experiment. In field tests and in growth chamber tests seeds were subsequently dried at room temperature for one day before sowing.

The pesticided Calthio C was applied in field tests as dry powder at 4 gram per kilogram of seeds up to one day before sowing. The fungicide, Bavistin was applied in seedling vigour test as dry powder at 2 g per kg seeds, one day before seeds were incubated for germination.

### Field experiments: Design andmanagement

Field trials were carried out from 2011-2013 on five different locations in Central Burkina Faso: At Farmers fields in four different villages(Kindi, Diapangou, Ipendo and Zorgo) and at the Kamboinsé Research Station near Ouagadougou. Two formats of field trials were conducted: Small plots in a random

complete block design (three plots per treatment on each field) withrows per plot, 5 m long; and large plots (a single plot per treatment on each field) with 20 rows, 30 m long. In all field tests, seeds were sown in rows with 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 15 days after sowing, the number of emerged seedlings per hole was reduced to a maximum of 4 seedlings. In all fields 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 applied 30 days post-emergence. Irrigation was not applied.3-4 weeks after sowing crop emergence was determined visually by counting the percentage of seed holes populated with emerging plants in each plot. At harvest, grain was collected from central rows of each plot (3 or 18 rows, dependent on plot size) and yield was calculated as kg $ha^{-1}$ as measured after 2 weeks of drying of grain at room temperature.

**Field experiments: Statistics**

For each treatment the average yield was calculated both in absolute numbers (kg/ha) and as a relative and normalized value, $\%^N$ as in Zida *et al*., 2012 (average of control normalized to $100\%^N$). Before normalizationacross all experiments in a trial, the yield of each treatment in each experimental set of plots (complete block design)was calculated relative to the average of all treatments in the same block. Statistical comparison between treatments in field-tests was made using one of the following tests: t-test, paired t-test and Wilcoxon paired test as indicated using software PAST version 2.1 (Hammer *et al*., 2001).

**Seedling vigour test**

The seedling vigour was determined based on 400 treated and untreated sorghum seeds in four replicates of 100 seeds each according to the between-paper method (ISTA, 2003).The seeds were treated with 2, 4, 6, 8 and 10% (w/v) concentration *E. alba* plant extract by soaking for 1, 2, 4, 6, 8, 10, 12 and 24 h. Treated and non-treated seeds were seeded onto paper towels soaked in distilled water. One hundred seeds were placed equidistantly on the paper towel and covered with another pre-soaked paper towel, and rolled up along with polythene wrapping to prevent drying of towels. The rolled towels were then incubated in incubation chamber at 24 ± 1°C for 10 days. After incubation, the towels were unrolled and seedling vigour was determined by measuring the length of the root and shoot of individual seedlings. The vigor Index was calculated using the formula from Baki and Anderson (1973):VI = (mean root length + mean shoot length) X (percent germination).

**Growth chamber test**

The assay testing fungal pathogenicity *in vitro*described by Leslie *et al*. (2005) was used with the following modifications: Seeds of sorghum from sample #49.071 (Kapélga Base, INERA, Burkina Faso) were used in all experiments.After treatment and drying of seeds as described above, the seeds were sown in 80 ml test-tubes (1 seed per tube) containing 20 ml solidified sterile Hoagland's plant growth medium no. 2. The plants were incubated in a climate chamber at 25 °/16 °C day/night temperature and a light/dark ratio of 14/10 h using a continuous photon flux density of 58–65 µmol s-1 m-1 photosynthetically active radiation (PAR) for 16 days after sowing. Plant growth was visually assessed 12 days after sowing by recording of germination, shoot height, root development (presence of primary root with lateral roots more than 1 cm long) and root health (presence or absence of dark necrotic tissue). At day 16 after sowing, shoot dry weight was measured after drying at 70 °C for three days. The assay consisted of six independent replicates with n=24 seeds per treatment. Means with 95% confidence intervals and statistical difference between inoculated and control groups were calculated using a t-test within the software PAST (Hammer *et al*., 2001). Similarity of binomial records of root development (normalor abnormal) and root health (presence or absence ofroot necroses) was tested by $\chi^2$-test, using the same software. Seeds for inoculation with *Epicoccum sorghinum* were heat treated in a water bath at 55°C for 40 min before inoculation in order to reduce the natural inoculum of seed borne fungi. Seeds were subsequently dried overnight before sowing in test tubes and inoculation with *Epicoccum sorghinum* ($2.5 \times 10^5$spores pr seed).

**Seed mycoflora**

The blotter method, as described by Mathur and Kongsdal (2003), was used to characterize seed mycoflora. In total, 400 seeds (8 replicates of 50 seeds) were placed on water-soaked filter paper in 9 cm Petri dishes and incubated for 7 days at 20 ± 2°C, under 12 h alternating cycles of near ultraviolet light and darkness. Subsequently, seeds were individually examined under stereo and compound microscopes for the presence of fungal infections.

## RESULTS

### Effect of seed treatment on seedling virgour *in vitro*

A combination of five different concentrations of *E. alba* extract (2-10%) and eight different soaking times (1-24 hours) were tested on seeds of sorghum and Vigour Index was measured after 7 days (Fig. 1). An almost uniform positive effect on seedling vigour was observed for treatments applying any of the five concentrations tested together with a soaking time of 6-10 hours. With a shorter soaking time (4 hours) an apparent dose response was observed for *E. alba* extract concentration (2% < 4 % < 6≈8≈10%) and an opposite response (10 < 8% ≈ 6% < 4% < 2%) was observed for soaking 12 hours. Treatment with fungicide clearly showed the strongest positive response indicating that seedling vigour was negatively affected by seed-borne fungi.

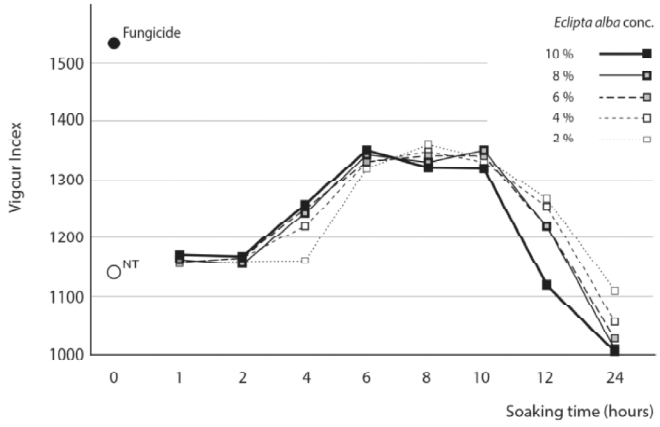


**Figure 1: Effect of *Eclipta alba* extract concentration and soaking time on seedling vigour *in vitro***

Virgour index of 7-day-old seedlings of sorghum was measured to compare the effect of different seed treatments. All combinations of five different concentrations of *Eclipt aalba* extract and eight different soaking times varying from 1 -24 hours were compard. NT = No Treatment. Fungicide = Bavistin (Carbendazim 50% WP).

### Lowering extract concentration in field experiments

During three growing seasons the application of high dose (10% *E. alba* extract) was compared to two lower concentrations (1.25% and 2.5%) and pure water (0%), respectively. A total of 24 field tests were performed in five different locations (Table 1). When yield was measured in absolute values (kg/ha) a yield increase of 13-26% was observed for all three concentrations of*E. alba* extract. When the yield increase was calculated as relative values for each individual field and subsequently normalized (Average of Dose_0% = 100), a significant 22-25%$^{N}$ yield increase was obtained for 2.5% and 10% *E. alba* extract ($p$<0.0038 and $p$<0.0078, respectively) (Table 1).

### Reducing soaking time

In an independent field trial five different soaking times in *E. alba* extract 12.5% (6h, 8h, 10h, 12h and 14h) were tested at four different trial locations in Burkina Faso. Comparison was made to dry seed treatment with Calthio C and No Treatment (Table 2). Reduced soaking time (6-8 hours) allowed an absolute yield increase of 155-353 kg/ha compared to 526 kg/ha observed for 10 hours. When calculated as relative values almost similar results were found for 6 h soaking compared to 10 h soaking (30.9 and 31.6 %$^{N}$, respectively). However, for 6 h soaking the yieldincrease was only border line significant ($p$<0.08) in contrast to 10h soaking ($p$<0.04).

Since neither the initial test on seedling vigour (Fig. 1) nor the test on yield in Table 2 was designed to

**Table 1**
**Effect of *E.alba* extract concentration on the yield of sorghum**

| | *Absoluteyield (grain)* | | | *Relative and normalized yield (%$^{N}$)* | | |
|---|---|---|---|---|---|---|
| *Dose* | *Yield kg/ha* | *Δyield Relative to Dose_0% kg/ha* | *Yield Relative to Dose_0% %* | *Yield* Relative to Dose_0% %$^{N}$* | *95% conf. %$^{N}$* | *p value** for similarity to Dose_0%* |
| 0 % | 945.4 | 0 | 100 | 100.0 | +/- 12.9 | NA |
| 1.25 % | 1072 | +126.6 | 113.4 | 111.9 | +/- 11.4 | 0.16 |
| 2.5% | 1100 | +154.6 | 116.4 | 125.2 | +/- 11.5 | 0.0038 |
| 10% | 1194 | +248.6 | 126.3 | 122.0 | +/- 10.0 | 0.0078 |

Twenty four field trials (N= 24) carried out at 5 different locations in the period 2011-2013.
Six different seed samples were included in the test in total. Soaking time 10 hours were used in all treatments.
*Yield (%$^{N}$) calculated relative to the average of all treatments within a block (set of field plots) and subsequently normalized (Dose_0% = 100%$^{N}$) across all blocks in the trial.
** t-test

**Table 2**
**Effect of soaking time on the yield of sorghum (12.5% *E. alba* extract)**

| | *Absolute yield* | | | *Relative and normalized yield (%$^N$)* | | |
|---|---|---|---|---|---|---|
| *Soaking time* | *Grain Weight kg/ha* | *Δyield Relative to NT kg/ha* | *Yield Relative to NT %* | *Yield* Relative to NT %$^N$* | *95% conf. +/- %$^N$* | *p value** for similarity to NT* |
| NT | 1120 | 0 | 100.0 | 100.0 | +/- 19.9 | NA |
| 6 hours | 1473 | +353 | 131.6 | 130.9 | +/- 21.4 | 0.08 |
| 8 hours | 1275 | +155 | 113.9 | 115.5 | +/- 14.0 | 0.29 |
| 10 hours | 1646 | +526 | 147.0 | 131.6 | +/- 16.1 | 0.04 |
| 12 hours | 1427 | +307 | 128.3 | 121.6 | +/- 13.8 | 0.13 |
| 14 hours | 1178 | +58 | 105.2 | 106.9 | +/- 10.2 | 0.60 |
| Calthio C | 1665 | +545 | 148.7 | 148.3 | +/- 11.4 | 0.001 |

Twenty five trials (N = 25) carried out at 4 different locations during 2012.
Five different samples of farm-saved seed were included. NT = No treatment
*Yield (%$^N$) calculated relative to the average of all treatments within a block (set of field plots) and subsequently normalized (NT = 100%$^N$) across all blocks in the trial.
** t-test

discriminate the effect of plant extract from the effectof hydropriming (soaking in pure water) we also tested the specificity of the plant extract at reduced soaking time (6h) by comparison to pure water (Table 3). By pairwise comparison with soaking of seeds for 6 h in water an average yield increase of 19.1%$^N$ was obtained for soaking in *E. alba* extract 10% w/v ($p$<0.012). Individual results for absolute yield (kg/ha) illustrated the large variation observed between individual fields.

### Combining low dose and reduced soaking time

During 2012 and 2013 a series of 19 field tests were conducted comparing doses of *E. alba* extract 2.5% and 10 % at 6 hours soaking time (Fig. 2). Field tests were carried out as demonstration tests (large plots) on Farmers fields in five different areas of Burkina Faso and as small plots on Kamboinsé Research Station. On an average, a 27%$^N$ yield increase (relative and normalized values) was obtained for low dose and a 13%$^N$ increase for high dose. For the low dose, the average yield differed significantly from yields obtained from non-treated seeds ($p$<0.0042 ; students t-test). In absolute values low dose caused a yield increase of 180 kg/ha (16%). Also crop emergence was significantly increased more than 7% by low dose treatment compared to non-treated seeds ($p$<0.005, Wilcoxon paired test). Treatment with Calthio C was included for comparison and a similar improvement of yield and crop emergence as observed for low dose plant extract was found for this treatment.

### Experiments in growth chamber

Seed treatment experiments comparing three doses of *E. alba* extract (2.5% – 5% – 10 %) at 6 hours soaking

**Table 3**
**Specificity of *E. alba* extract (6h soaking time) on the yield of sorghum**

| | | *Absolute yield kg/ha* | | *Relative and normalized Yield %$^N$* |
|---|---|---|---|---|
| *Experiment No.* | *Water kg/ha* | *E.a. 10% kg/ha* | *E.a. 10% ΔYield relative to water kg/ha* | *E.a. 10% ΔYield relative to water %$^N$* |
| 1 | 2674 | 2794 | + 120 | +4.5 |
| 2 | 1238 | 1399 | + 161 | +13.0 |
| 3 | 2859 | 3218 | + 359 | +12.5 |
| 4 | 1984 | 2028 | + 44 | +2.2 |
| 5 | 531 | 752 | + 221 | +41.6 |
| 6 | 777 | 1195 | + 418 | +53.9 |
| 7 | 250 | 265 | + 15 | +5.8 |
| 8 | 273 | 325 | + 52 | +18.9 |
| Average | 1323 | 1497 | + 174 (13.2%) | +19.1 %$^N$ |
| *p*- value* | | | 0.012 | 0.012 |

Experiments made during 2013 on five locations using one seed sample (INERA, Kapelga)
*Likelihood of similarity between treatment with *E.a.*10% and water; Wilcoxon paired test

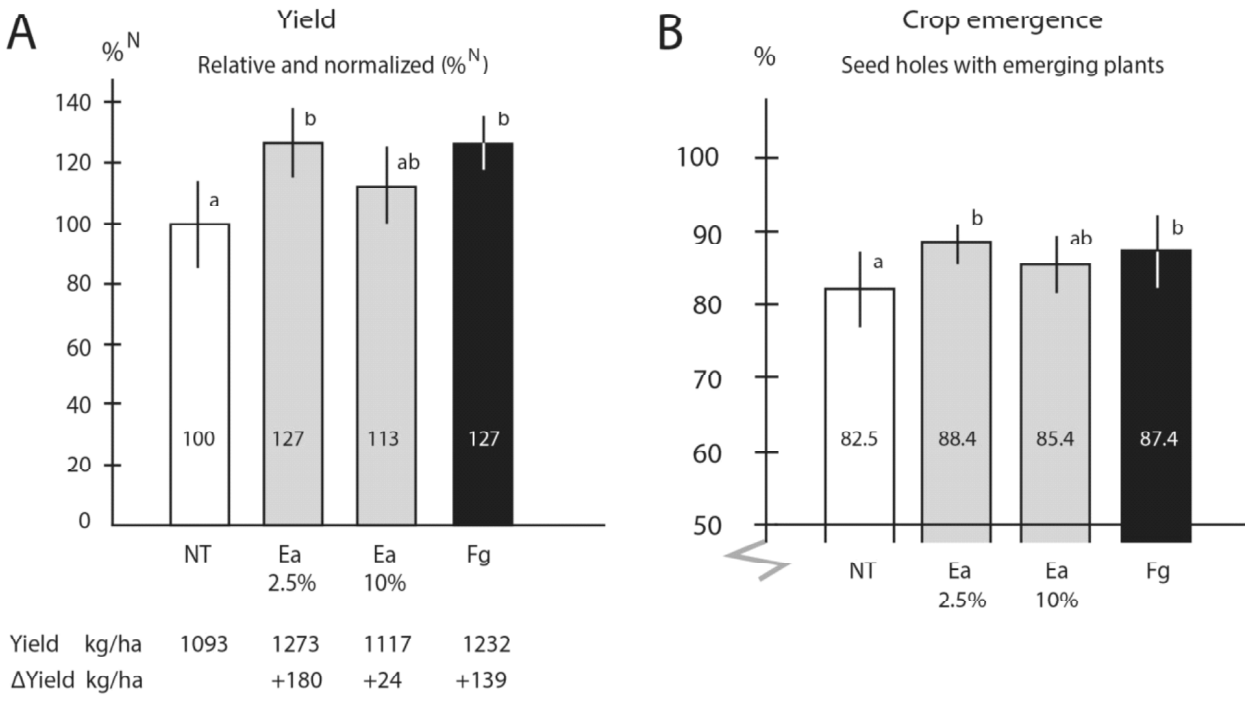


**Figure 2: Field trial of high and low doses of *E. alba* extract at 6 h soaking time**

Effect on yield (A) and emergence (B) by high dose (10%) and low dose (2.5%) of *Eclipta alba* extract applied in seed treatment using 6 hours of soaking time. Nineteen field tests of sorghum (N=19) were carried out from 2012-2013 in five different locations. A total of four different seed samples were tested. Fg = fungicide (Calthio C). Columns with different indices are significantly different ($p<0.005\%$) using *t*-test (A) and Wilcoxon paired test (B).

were performed in a synthetic, transparent growth medium in test tubes (agar with plant nutrients). Two controls were included: no treatment (NT) and soaking in pure water ($H_2O$). A regular sample of sorghum seeds produced commercially in Burkina Faso was chosen for the experiments. In this system treatment with 2.5% and 5% *E. alba* extract produced a significant increase in shoot dry weight (28% and 34% respectively) compared to non-treated seeds (Fig. 3). Compared to seeds treated with pure water the increase in shoot dry weight was even larger. In both non-treated seeds and in seeds treated with water a high frequency (33-39%) of plant roots showed symptoms of pathogenic fungal infection in the form of dark, necrotic tissue. This frequency was reduced more than three fold in all treatments with *E. alba* extract ($p<0.001$; $\chi^2$-*test*). At the same time the frequency of plants with secondary roots was found to increase for the groups of plants treated with *E. alba* extracts (Fig. 3).

A blotter test on 400 seeds of the sample used for growth chamber experiments above, revealed that seed infection by *Epicoccum sorghinum* and *Curvularia* spp. amounted to 30.3% and 29.5%, respectively (Fig 4A). Seed infection by other pathogenic fungi such as *Fusarium spp.*, *Exserohilum rostratum* and *Colletotrichum graminicola* was below 5% in total. Inoculation of heat treated seeds with spores of *Epicoccum sorghinum* clearly showed that both pathological phenotypes observed above, dark necrotic root tissue and reduced formation of secondary roots, could be associated with the inoculation with *E. sorghinum* (Fig. 4B). In contrast, no formation of dark root necrosis was observed in plants from non-inoculated and heat treated seeds.

## DISCUSSION

### Summary of findings

In this study we have tested the potential for increasing the feasibility of a seed treatment procedure involving the soaking of sorghum seeds in an aqueous extract of the plant *E. alba*. Compared to non-treated seeds, a significant increase of crop yield was obtained using a combination of low amount of *E. alba* plant material for extraction (2.5% w/v) and a reduced soaking time for seeds (6 hours). In comparison to previously published methodology (concentration of plant extract ≥10%; soaking time ≥10 h) we find a potential for using less plant material

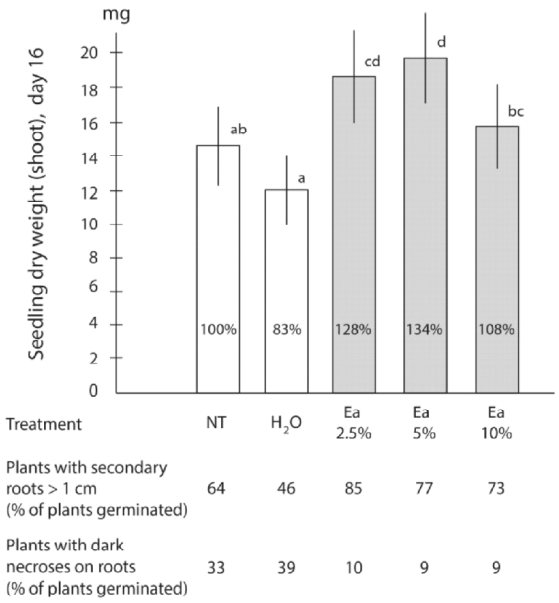


**Figure 3: Growth and health of sorghum seedlings (growth chamber) for different concentrations of *E. alba* extract in seed treatment**

Seeds were germinated and grown for 16 days in tubes with agar and growth medium Hoaglands No. 2. For each of five seed treatments, three different parameters were monitored: 1) Shoot dry weight (columns), 2) % of plants with normal root development (formation of secondary roots longer than 1 cm) and 3) % of plants with dark necrotic lesions on roots. For each treatment 144 seeds were tested. Columns with different indices (a, b, c, d) are significantly different ($p$<0.05 ; $t$-test). 95% confidence intervals are indicated by thin bars

and less time in order to obtain a yield increase. Consistently, seedling vigour-, growth- and health-promoting effects of the combination of low concentration of plant extract and reduced soaking time were demonstrated *in vitro*.

**Results *in vitro***

In the present study the effect of soaking seeds in water (1-24 hours)with different concentrations (2-10% w/v) of plant extract was assessed in a 7-days assay. An upper plateau in the effect on seedling vigour was observed for all concentrations tested using soaking times between 6 and 10 hours. The observed effect represents the combined effect of compounds extracted from the plant *E. alba* and hydropriming (exposure to water before sowing). Hydropriming has previously been reported to have a positive effect on sorghum emergence and vigour index using soaking times between 6 and 10 hours (Harris 1996, Shehzad 2012) which appears very consistent with the present findings. In seeds of sorghum, water uptake (imbibition) after 6 and 10 hours soaking at 25° C have been reported to be 66% and 72%, respectively, compared to total uptake measured after 48 hours (Al-Mudaris and Jutzi 1998). Thus, already after 6h a considerable amount of water has been absorbed in the seeds and this could influence results substantially – particularly in a short term assay. However, when the effect of seedling

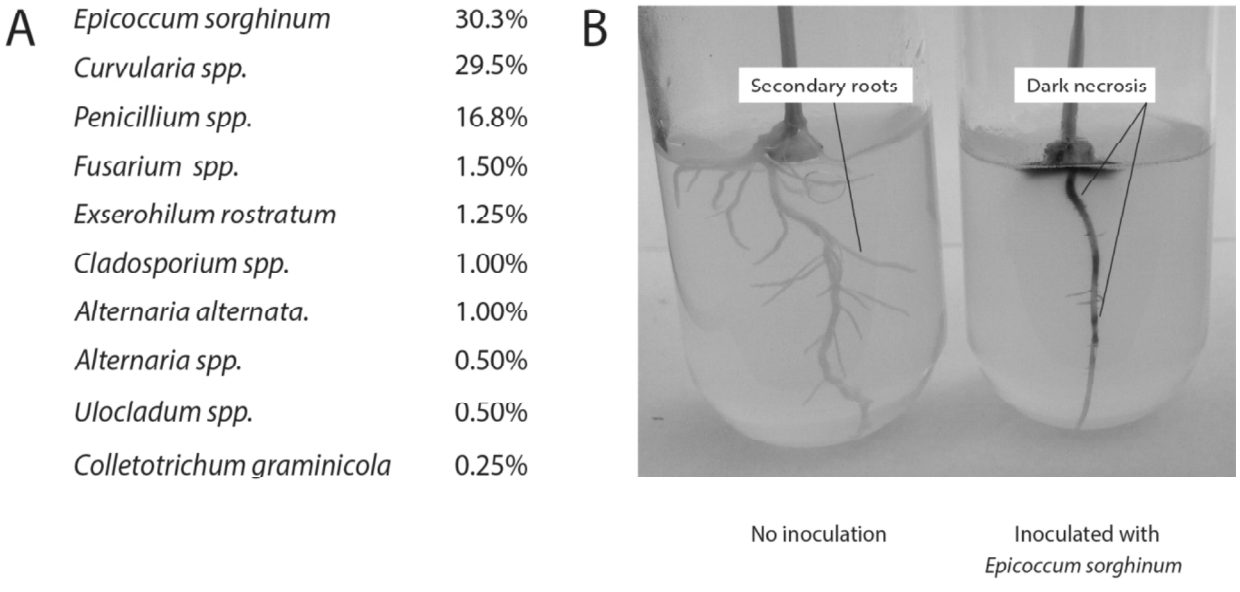


**Figure 4: Seed mycoflora and fungal inoculation of heat treated seeds of sorghum**

(A) Seed mycoflora: Blotter test showing percentage of seeds infected by different fungal species after incubation for seven days. (B) Fungal inoculation : Seedlings grown in nutritional agar. The plant to the right was grown from a seed inoculated with spores of *Epicoccum sorghinum* . All seeds were heat treated before sowing to eliminate the natural mycoflora.

growth was tested in a 16-days assay (growth chamber) we found that the growth and plant health promoting effect was attributed to the plant extract *per se*, since hydropriming alone (soaking in pure water) did not produce a positive effect neither on shoot dry weight nor on root health (formation of secondary roots, absence of necroses). It is very likely that the effect exerted by the plant extract partly depends on the microflora found in the seeds tested. In the blotter test, the seeds were found to contain a high infection level (≈30%) of both *Epicoccum sorghinum* and *Curvularia spp*. which is commonly observed in Burkina Faso (Zida *et al*. 2008a). One effect found for the plant extract was to reduce the frequency of root necrosis observed in roots from 39% (observed in hydroprimed seeds) to 9-10% in plants treated with extract. At the same time the frequency of roots having formation of secondary roots increased from 46% to 73-85%. Both observations are consistent with an inhibitory effect of *E. alba* extract on transmission of seed-borne, pathogenic fungi such as *E. sorghinum* for which symptoms are shown in Fig.4B.We previously have found *E. sorghinum* being susceptible to the extract of *E. alba* in a dose dependent manner (Zida *et al*. 2012).

**Results from field experiments**

Previously, an average of 17-18%$^{N}$ yield increase (≈150 kg/ha) was found for seed treatment with *E. alba* extract at high dose (conc ≥10%; soaking time ≥ 10 hours) in farmers field relative to either no treatment or hydropriming, respectively (Zida *et al.* 2012). Here we find that a similar yield increase (≈150 kg/ha) could be obtained using either a low plant extract concentration (2.5%; $p<0.0038$) or by reducing soaking time from 10 to 6 hours ($p<0.08$). For reduced soaking time the observed yield increase was less significant (compared to non-treated seeds). However, in the subsequent test, soaking time of 6 hours in plant extract resulted in an average yield increase of 174 kg/ha compared to seeds treated with only water ($p<0.012$) (Table 3). When the combination of low concentration (2.5%) and low soaking time (6h) was subsequently tested, we found a relative yield increase of 27%$^{N}$, which was significant ($p<0.05$). In absolute numbers, the corresponding yield increase found was 180 kg/ha (16%). A similar yield increase was obtained using the binary pesticide Calthio C in the same trial, which corresponds to what has previously been reported for this pesticide (Zida *et al*. 2012). Somewhat surprising, the effect of high dose (10% - 6h) on yield was in this case rather weak (13%$^{N}$) and as in the previous experiment with different soaking times not significantly different from non-treated seeds. However, all together, the field data obtained demonstrates that it is possible to obtain a yield increase using four times less plant material for extraction and four hours less time for soaking of seeds compared to previous reports. However, we do not conclude that soaking in 2.5% w/v extract for six hours *per se* represents an optimal treatment in comparison to other combinations, nor that the effect observed in this study consistently will be observed under different agro-ecological conditions- orfor different types of sorghum seed. In particular, more field data on different soaking times in the interval of 6-10 hours could be justified. However, in areas of Burkina Faso where *E. alba* is commonly found, our results indicate that a minimum of 6 h soaking of seeds in a minimum of 2.5% w/v *E. alba* extract could be recommended as a resource-efficient and environmentally friendly seed treatment of sorghum. Several studies have shown the ability of different plant extracts to reduce fungal inoculum in seeds and/or seed-borne diseases in sorghum(Tegegne and Pretorious 2007, Raghavendra *et al*., 2007, Zida *et al*., 2008b, Wulff *et al*., 2011, Singh and Garampalli 2012, Bonzi *et al*., 2012, Manjunatha *et al*., 2013). However, to our knowledge, no other aqueous plant extract has so far shown similarly promising results in field trials of seed treatment using naturally infected seeds as here shown for the extract of *E. alba*. For a recent review of plant extracts and other non-chemical methods used in protective seed treatment see Koch and Roberts (2014).

**Future research**

The field trial testing the combination of reduced extract concentration and soaking time (Fig.2) did not discriminate between the effect of hydropriming and effects caused by compounds extracted from *E. alba* since treatment with pure water was not included as a control. Hydropriming alone has previously been reported to cause yield increases in sorghum field trials: 9.5% increase (10 hours soaking time ; Ramamurthy *et al*., 2005) and up to 85% increase (8 hours of soaking; Aune and Ousman 2011). In the latter case, the increase in yield was observed only when fertilizer was applied. In experiments presented in the present study and in Zida *et al*. 2012 fertilizer has been applied in all field tests. From our data, we cannot confirm nor exclude that hydropriming contributes to the observed yield increase. However, when tested separately both the low concentration of

plant extract (2.5%) and the reduced soaking time (6h) allowed a positive effect of the plant extract to be found compared to treatment with pure water alone in field trials (Table 1 and 3, respectively). One question to address in future research could be the influence of seed mycoflora in relation to the effect of hydropriming (6-10 h) and the effect of *E. alba* extract, respectively. Particularly it could be interesting to address the differences between seed samples with high and low levels of*E. sorghinum* infection. This fungus is known to be susceptible to the extract and was present at a high level in the seed sample here used to evaluate plant growth *in vitro* showing a very clear difference between the effect of hydropriming and treatment with the plant extract (Fig. 3).

If the technology of seed treatment using *E. alba* extract is going to be implemented by farmers it will also be important to know if a yield increase can be expected without the need of other agrochemical inputs. Therefore, another important question needing further investigation is the significance of the use of fertilizer with regard to the effect of seed treatment as reported for hydropriming by Aune and Ousman 2011.

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## REFERENCES

Al-Mudaris, M.A. and Jutzi, S.C. (1998), The Influence of Genotype, Priming Material,Temperature and Osmotic Potential of Priming Solution on Imbibition and Subsequent Germination of Sorghum and Pearl Millet Seeds During and After Treatment. Der Tropenlandwirt, Beiträge zur tropischen Landwirtschaft und Veterinärmedicin, 99, Suppl.:133-145.

Aune, J.B. and Ousman, A. (2011), Effect of seed priming and micro-dosing of fertilizer on sorghum and pearl millet in Western Sudan. Experimental Agriculture 47: 419-430.

Baki, A.A. and Anderson, J.P. (1973), Vigour determination in soybean seeds by multiple criteria. Crop Science 13: 630-633.

Bonzi, S.; Somda, I.; Zida, E.; Sereme, P. (2012), Efficacy of plant extracts and effect of seed soaking duration on treatment of sorghum seed naturally infected by *Colletotrichum graminicola* and *Phoma sorghina*. Arch Phytopathol Plant Protect 45:1405-1410.

Bonzi, S.; Somda, I.; Sereme, P.; Adam, T. (2013), Efficacy of essential oils of Cymbopogon citradus (D.C.) Stapf, Lippia multiflower Moldenke and hot water in the control of seed-borne fungi *Phoma sorghina* and their effects on *Sorghum bicolor* (L.) Moench seed germination and plants development in Burkina Faso. Net J Agricultural Sci 1:111-115.

Borkataky, M.; Kakoty, B.B.; Saikia, L.R. (2013), Proximate analysis and antimicrobial activity of *Eclipta alba* (L.) Hassk. – a traditionally used herb. Int J Pharmacy and Pharmaceutical Sci 5:149-154.

Dicko, M.H.; Gruppen, H.; Traoré AS, Voragen, A.G.J.; van Berkel, W.J.H. (2006), Sorghum grain as human food in Africa: relevance of content of starch and amylase activities. African J. Biotechnology 5:384-395.

FAO (Food and Agriculture Organisation of the United Nations) (2014),*Sorghum bicolor* (L.) Moench *http://www.fao.org/ag/agp/agpc/doc/gbase/data/pf000319.htm* (web page cited 1. August 2015).

Frederiksen, R.A. (2000), Diseases and disease management in sorghum. In *Sorghum*: Origin, History, Technology and Production. (Eds.: Smith, C.W. and Frederiksen, R.A.). Published by John Wiley & Sons, Inc. (New York). pp 497-533.

Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. (2001), PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1): 9pp.

Harris, D. (1996), The effects of manure, genotype, seed priming, depth and date of sowing on the emergence and early growth of *Sorghum bicolor* (L.) Moench in semi-arid Botswana. Soil & Tillage Res 40:73-88.

Hasan, H.A. ( 2001), Phytotoxicity of pathogenic fungi and their mycotoxins to cereal seedling viability. Mycopathologia 148 (3): 149-55.

ISTA, (2003),International Rules for Seed Testing. Proceedings of the International Seed Testing Association, Seed Sci Technol21:25-30.

Kaurivi, J.Z.U. and Shilulu, I. (2007), Priming sorghum seeds with water at different time rates. African Crop Science Conference Proceedings 8: 1689-1691.

Koch, E. and Roberts, S.J. (2014), Non-chemical seed treatment in control of seed-borne pathogens. *In*: Global perspectives on the health of seeds and plant propagation material (Vol 6) *Eds*.: Gullino ML, Munkvold G. ISBN 978-94-017-9388-9 Springer Dordrecht, Germany.

Leslie, J.F.; Zeller K.A.; Lamprecht, S.C.; Rheeder, J.P.; Marasas, W.F.O. (2005), Toxicity, pathogenicity, and genetic differentiation of five species of Fusarium from sorghum and millet. Phytopathology 95:275-283.

Majunatha, H.P.; Singh, H.; Chauhan, J.B.; Zameer, F.; Garampalli, R.H. (2013), Induction of resistance against sorghum downy mildew by seed treatment with *Duranta repens* extracts. J Agric Vet Sci 3: 37-44.

Mathur, S.B. and Kongsdal, O. (2003), Common Laboratory Seed Health Testing Methods for Detecting Fungi. ISTA, Bassersdorf, CH-Switzerland.

Mathur, S.B.; Njala, M.; Kongsdal, O. (2003), An illustrated handbook on normal and abnormal seedlings of tropical and sub-tropical crops. Danish Government Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark. ISBN 87-9898833-1-8.

Peraman, M.K.; Ramalingam, P.; Sai, B.J.N.N. (2011), Anti-inflammatory and antimicrobial activity of the extracts of *Eclipta alba* leaves. Eur J Exp Biol 1:172-177.

Raghavendra, V.B.; Lokesh, S.; Govindappa, M.; VasanthKumar, T. (2007), Dravya – As an organic agent for the management of seed-borne fungi of sorghum and its role in the induction of defence enzymes. Pesticide Biochemistry and Physiology 89: 190-197.

Ramamurthy, V.; Gajbhiye, K.S.; Venugopalan, M.V.; Parhad, V.N. (2005), On-farm evaluation of seed priming technology in sorghum (*Sorghum bicolor L*). Agricultura Tropica et Subtropica 38: 34-40.

Rooney, L.W. (1996), Sorghum and millets. In *Cereal grain Quality* (Eds.: R.J. Henry and P.S. Kettlewell) published by Chapman & Hall.

Saraswathy, N. and Kumaran, P.M. (2012), Evaluation of aqueous extract of *Eclipta alba* leaves for preservative potential against *Fusarium* species. Am J Pharmtech Res. 5:647-649.

Shehzad, M.; Ayub, M.; Ahmad, A.U.H.; Yaseen, M. (2012), Influence of priming techniques on emergence and seedling growth of forage sorghum (*Sorghum bicolor* L.). The J. Animal & Plant Sci. 22:154-158.

Singh, H. and Garampalli, R.H. (2012), Screening of plant extracts for organic management of downy mildew of sorghum. Arch Phytopathol Plant Protection 45:1732-1740.

Tarr, S.A.J. (1954), Protection of sorghum against soil fungi, soil pests and covered smut by combined insecticide-fungicide seed dressings. Ann. Appl. Biol 41:578-585.

Teetes, G.L. and Pendleton, B.B. (2000), Insect Pests of Sorghum.In *Sorghum*: Origin, History, Technology and Production. (Eds.: Smith, C.W. and Frederiksen, R.A.). Published by John Wiley & Sons, Inc. (New York). pp 443-495.

Tegegne, G. and Pretorius, J.C. (2007), *In vitro* and *in vivo* antifungal activity of crude extracts and powdered dry material from Ethiopian wild plants against economically important plant pathogens. BioControl 52: 877-888.

Wulff, E.G.; Zida E.; Torp, J.; Lund, O.S. (2011), *Yucca schidigera* extract: a potential biofungicide against seedborne pathogens of sorghum. Plant Pathol. 61: 331-338.

Zida, E.P.; Sérémé, P.; Leth V.; Sankara, P. (2008a), Effect of aqueous extracts of *Acacia gourmensis* A, Chev and *Eclipta alba* (L.) Hassk. On seed health, seedling vigour and grain yield of sorghum and pearl millet. Asian J Plant Pathol. 2:40-47.

Zida, P.E.; Sérémé, P.; Leth, V.; Sankara, P.; Somda, I.; Adama, N. (2008b), Importance of seed-borne fungi of sorghum and pearl millet in Burkina Faso and their control using plant extracts. Pakistan J Biological Sciences 11:321-331.

Zida, P.E.; Lund, O.S.; Néya, J.B. (2012), Seed treatment with a binary pesticide and aqueous extract of *Eclipta alba* (L.) Hassk. for improving sorghum yield in Burkina Faso. *J Tropical Agriculture* 50, 1-7.