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Seed treatment with *Eclipta alba* plant extract and application of fertilizer confers a combined effect on the yield of sorghum

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Abstract

A potential to improve yield of sorghum has previously been demonstrated using seed treatment with an antifungal extract from the plant, *Eclipta alba*. The objective of the present study was to compare the effect of the extract when applied with and without the use of fertilizer (NPK and urea). Over two years, seventeen field tests were carried out on nine farms in South-Eastern Burkina Faso. The farms were characterized by a low baseline yield of sorghum (<400kg/ha) and an annual precipitation of 800-900 mm. The seeds used for testing carried a natural inoculum of fungal pathogens including *Epicoccum sorghinum* and *Curvularia* spp. as commonly observed in the region. The plant extract alone provided a yield increase of +21% as a field average ($p<0.05$). Fertilizer alone provided a mean yield increase of + 23% ($p<0.02$). The combined use of plant extract and fertilizer resulted in a mean yield increase of 66% indicating at least an additive effect ($p<0.0003$) and a possible, positive interaction (synergism; $p<0.11$). With respect to emergence, an additive effect was observed but it was not significant. Compared to the treatment with plant extract, hydropriming only provided moderate effects. In conclusion, the use of plant extract and fertilizer in combination showed a superior effect on yield. Additional field data supporting the choice of extract concentration (2.5% w/v) and the effect on formal and informal sources of seeds are provided.

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Introduction

In Western Africa, seeds of sorghum are commonly infested by a range of phytopathogenic ascomycetes (Nutsugah *et al*., 2004; Zida *et al*., 2008a; Stokholm *et al*., 2016). As an inexpensive resource available to farmers, several extracts from African plants have been found to control seed-borne pathogens in sorghum (Review by Koch and Roberts 2014). In Burkina Faso, emergence and yield of sorghum have been found to increase when an antifungal extract derived from the invasive weed and medicinal plant *Eclipta alba* is used in seed treatment (Zida *et al*., 2012 and 2018). The previous experiments testing *E. alba* extract were all carried out on field plots receiving mineral fertilizer. However, the use of mineral fertilizer is not common among West African farmers growing sorghum. The aim of the present study was therefore to compare the effect of seed treatment in the presence and in the absence of fertilizer.

Several field experiments using a high concentration (>10% w/v) of *E. alba* extract have previously shown, that the effect on yield varies between locations in Burkina Faso (Zida *et al*., 2016). It was recently shown, that a significant effect on yield can be obtained using a lower concentration (2.5% w/v) of *E. alba* extract (Zida *et al*., 2015). Before testing the interaction of fertilizer and seed treatment (main objective) we therefore conducted experiments with the objective of identifying optimal experimental conditions (field location, extract concentration).

Soaking of sorghum seeds in pure water (hydropriming) before sowing has previously been shown to interact synergistically with the use of fertilizer (Aune and Ousman, 2011; Abdalla *et al*., 2015). In the main experiment testing interaction of plant extract and fertilizer we therefore included hydropriming as a treatment for comparison to the plant extract. As a pre-requisite for implementing the seed treatment technology using *E. alba* extract by farmers, we finally also conducted a participatory comparison of the effect of plant extract applied to farm-saved seeds and formally propagated seeds, respectively.

Materials and methods

Seeds

For field trials with formally produced seeds in 2015, 2016 and 2017 seed lots of varieties Kapelga and Kouria (both propagated by IN.E.R.A, Burkina Faso) were used as indicated in tables. On each field the same variety was used in all plots. Natural mycoflora in seed samples was assessed for the years 2016 and 2017 (Table 1). For field trials with farm-saved seeds in 2016, seed samples from each of 6 farmers near the villages Diapangou and Nakoaléongo were used (South-Eastern region of the country).

Table 1. Natural fungal inoculum in formally produced seed samples.

Year	Variety	Percentage of seeds infected (%)*				
		Curvularia spp.	*Epicoccum sorghinum*	*Fusarium* sp.	*Cladosporium* sp.	*E. rostratum*
2016	Kapelga	41	67	28	8	2
	Kouria	35	75	31	10	4
2017	Kapelga	50	15	6	5	4
Average	-	42	52	22	8	3

*Measured for 200 seeds using Blotter-test (Mathur and Kongsdal, 2003).

Plant extract and seed treatment

Wild plants of *E. alba* were collected in Central Burkina Faso, stored and extracted as previously described for *E. alba* extract 2.5% w/v (Zida *et al*., 2015). Shortly, extraction of *E. alba* powder in distilled water for 20 hours was carried out at 25-30 °C before use. Seeds were either not treated (NoT), soaked for 6 h in distilled water (H_2O) or soaked for 6 h in *E. alba* extract (Ea 2.5%). Seeds were then dried over night before sowing.

Field experiments

Experimental plots were prepared and managed as described in Zida *et al*., 2016. Shortly, for each treatment an experimental plot of at least 25 rows (80cm spacing, 5m length) was prepared (one plot per treatment per field) in a random block design. On "fertilized plots" mineral fertilizer (NPK 14-23-14, TOGUNA AGRO INDUSTRIES, Mali, 100kg/ha) was applied at the time point for sowing. Four weeks after sowing, Urea (OMNIFERT, Ghana, 50kg/ha, 46-0-0) was applied. Sowing was done in May-June and harvesting was done in November-December according to local weather forecasts and as previously described (Zida *et al.*, 2016).

Statistics

For each plot, the yield of grain was calculated in two ways: Absolute yield =kg/ha and a field average (%) calculated relative to the average yield of all plots of all treatments in the same block (field). Simple means of both types of yield were calculated. One-way ANOVA was calculated based on field averaged yields using software PAST version 3.20 (Hammer *et al.*, 2001). For all data sets showing an overall significant one way ANOVA p-value (<0.05) a pairwise comparison of each group was done by Mann-Whitney analysis using the same software (no requirement of a normal distribution of data). Two-way ANOVA to detect interaction between plant extract and fertilizer was made by grouping 17x4 = 68 plots from field trial 2016-2017 into ["NoT" OR "Ea2.5"] and ["Minus Fertilizer" OR "Plus fertilizer"], respectively. Plots with hydropriming were excluded from this analysis.

Results and discussion

Field locations and extract concentration

Before initiating experiments on the effect of fertilizer, a series of experiments was conducted to test how the previously developed protocol using a low concentration (2.5%) of *E. alba* extract was performing compared to high extract concentration (10%) in four locations (villages, Table 2). Three of the locations were selected based on previous positive results obtained with high concentration of plant extract (Zida *et al.*, 2016 and 2018). The fourth location, Nakoaléongo was selected due to its proximity to the village Diapangou in a region where the plant *E. alba* is very common (Zida *et al.*, unpublished survey). All four locations are located in sorghum producing areas of Central and South-Eastern Burkina Faso.

As expected from previous experiments, a large variation in the mean baseline yield levels was observed between locations/villages (from 292 to 1555kg/ha for No Treatment, Table 2). For the same reason, statistics was made on field averaged values (%) in order to avoid a bias from high yielding fields. Across all locations (Total) no significant difference was found between low and high concentration of plant extract. Based on this, and in order to obtain maximum feasibility of the protocol we decided to use the low concentration of extract in all subsequent experiments. In the two closely located villages, Diapangou and Nakoaléongo, a strong, positive effect of the low concentration of extract was obtained (>80% yield increase as a field average in both locations, Table 2). The area near these two villages was subsequently chosen for experiments testing the effect of +/- fertilizer. Note that the average baseline yield (No treatment) in the two villages was very low (<400kg/ha) despite an annual precipitation above 800 mm measured at Diapangou (Table 2).

Table 2. Comparison of low (2.5%) and High (10%) conc. *E. alba* extract on twenty fertilized fields (2015).

Village	Location	Rain* (mm)	N	Mean yield (kg/ha)			Mean yield (Field average%)		
				NoT	Ea2.5%	Ea10%	NoT	Ea2.5%	Ea10%
Kamboinse	N:12⁰26´ W:1⁰33´	901	5	1555	2465	2222	74.9	121.7	103.3
Ipendo	N:12⁰1´ W:2⁰19´	-	8	1056	1056	1118	97.9	98.4	103.8
Nakoaléongo	N:12⁰7´ W:0⁰11´	-	4	354	711	673	60.3	123.1	116.6
Diapangou	N:12⁰7´ W:0⁰11´	851	3	292	526	615	59.5	109.7	130.8
Total **			**20**	**926**	**1260**	**1230**	**78.9[a]**	**111[b]**	**110[b]**
95% conf.				na	na	na	+/-10.1	+/-7.7	+/-7.9

N = Number of field experiments; NoT = No Treatment; Ea = *Eclipta alba* extract; na = not applicable.

In all plots variety Kapelga was used and mineral fertilizer was added.

* Annual rain fall May-October measured in two out of the four locations

**Columns with same letters are not significantly different (Mann-Whitney paired test, p<0.05).

Effect of fertilizer and seed treatments on yield

A two-year field trial was conducted near the villages Diapangou and Nakoaléongo to compare the effect of seed treatments in the presence and absence of fertilizer, respectively. A total of seven-teen field tests were conducted (Table 3). Overall, the differences of yield between the six treatments were highly significant (one-way ANOVA: p<0.00001) and several pair-wise differences were subsequently identified as significant (Table 3, Tot. Field Av. (%)).

Effect of fertilizer alone

For each of the three treatments (NoT, H_2O, Ea2.5) the field averaged values were significantly higher in fertilized compared to the corresponding treatment in non-fertilized plots (Table 3). Similarly, a two-way ANOVA (comparing Minus and Plus fertilizer) was significant ($p<0.05$; not shown in table). For Non-treated seeds (NoT) the mean effect of fertilizer was a yield increase of approximately +23% (97.6 relative to 79.4). At least two previous studies in the region have obtained substantially stronger responses of the use of fertilizer: 80-81% yield increase was found for two varieties of sorghum in Burkina Faso using NPK and urea (Palé *et al.*, 2009) and +48% yield increase was observed for sorghum in Ghana using only urea (Buah *et al.*, 2012). The relatively weak response observed in the present study combined with the low yield levels (<450kg/ha; NoT in the presence of fertilizer) could indicate that other factors than nutrients were acting as yield-limiting factors on the fields selected for experiments.

Table 3. Effect of seed treatments and fertilizer on yield.

Trial year	N	Yield (mean)kg/ha						ANOVA One-way
		Minus fertilizer			Plus fertilizer			
		NoT	H_2O	Ea2.5	NoT	H_2O	Ea2.5	
2016	9	361	435	473	378	457	568	
2017	8	294	332	395	424	528	676	
Tot. (kg/ha)	17	330	387	436	399	491	619	
Tot. Field Av. (%)*	17	**79.4**^{a}	**87.4**^{ab}	**95.4**^{bc}	**97.6**^{bc}	**108**^{c}	**132**^{d}	<0.000001
95% conf.		+/-10.7	+/-11.3	+/-12.0	+/-9.7	+/-15.9	+/-14.8	

N = number of fields, NoT = No Treatment, H_2O = hydropriming, Ea2.5 = *Eclipta alba* 2.5%

Variety Kapelga was used in all plots.

* Columns with same letters are not significantly different (Mann-Whitney paired test, $p<0.05$).

Effect of E. alba extract alone

Without fertilizer a significant yield effect of Ea2.5% was found compared to NoT: +21% yield increase (Table 3: 95.4 relative to 79.4; $p<0.05$). All previously published results from field experiments testing seed treatment of sorghum with *E. alba* extract have included the application of fertilizer (NPK and urea) as a standard (Zida *et al.*, 2008b, 2012, 2015, 2016 and 2018). The finding of a significant effect on yield without any fertilizer used is, to our knowledge, thereby novel for the *E. alba* extract. Compared to hydropriming (H_2O) a stronger effect of the plant extract was observed both with and without fertilizer in both years (Table 2). The "additional" effect of the plant extract compared to pure water might be caused by the antifungal effect previously shown for the extract (Zida *et al.*, 2018) since the seeds samples used for testing all contained a substantial natural inoculum of the fungal genera *Epicoccum* and *Curvularia* (Table 1) known to include common, seed-borne pathogens of sorghum (Prom, 2004; Stokholm *et al.*, 2016).

Combined effect on yield

The combination of plant extract and fertilizer resulted in a yield significantly higher than all other treatments: 66% yield increase compared to NoT without fertilizer (Table 3: 132 relative to 79.4, $p<0.05$). This was significantly more than the effect of the individual treatments (95.4 and 97.6) indicating an additive effect ($p<0.0003$) and substantially more than the sum of the two individual effects (66% >> 21% + 23%) indicating a potential synergism. However, the potential synergism (interaction) between "Ea 2.5%" and "Fertilizer" was only borderline significant when tested by two-way ANOVA (p<0.11; not shown in table).

A synergistic interaction between seed hydropriming and micro-dosing of fertilizer was previously reported in sorghum (Aune *et al.*, 2011) and a strong combined effect on sorghum yield (+86%) was found for a combination of three treatments: seed hydropriming, soil fertilizing and treatment of seeds with pesticide Apron (Aune *et al.*, 2012). The latter study was carried out in neighbouring country, Mali, at similar latitude (Village Kanja, 12.3° N) as in the present study and similarly close to the 900mm isohyet. Also in Mali a yield increase of 47% was recently demonstrated by combining hydropriming, micro-dosing of fertilizer and mechanization (Aune *et al.*, 2018). Thus, a combined effect of seed treatments and fertilizer have previously been reported in Western Africa and the findings reported here are adding the plant extract from *E. alba* as an option for similar integrated crop management (ICM) in sorghum.

Effect on emergence

With respect to emergence the effects of treatments were less significant than the corresponding differences in yield (Table 4). However, a significant effect was found both for the plant extract (102.0 compared to 91.8) and for addition of fertilizer (101.7 compared to 91.8). As found for the yield, the strongest increase of emergence was observed when the two treatments were combined (108.9 compared to 91.8) all though it was not significantly different from the individual treatments. The finding of a significant effect of fertilizer alone on emergence is in agreement with results from a recent field study conducted in Zimbabwe (Rurinda *et al.*, 2014) in which a strongly positive effect on emergence was found for sorghum and finger millet, but not for maize. Several older studies testing the effect of fertilizer on emergence in a range of grasses and cereals (other than sorghum and millets) did not find any positive effect of fertilizer on emergence (Nyborg, 1961; Welch *et al.*, 1962). Thus, sorghum and millet might differ from other cereal crops in this regard. The observation of a positive effect on emergence by the plant extract alone (also compared to pure water) is in agreement with our previous findings (Zida *et al.*, 2018).

Table 4. Effect of seed treatments and fertilizer on emergence.

Trial year	N	Emergence (mean)%						ANOVA One-way
		Minus fertilizer			Plus fertilizer			
		NoT	H_2O	Ea2.5	NoT	H_2O	Ea2.5	
2016	9	61.8	59.8	68.2	65.1	66.2	71.1	
2017	8	66.3	73.8	73.5	75.9	75.0	79.3	
Tot.%	17	63.9	66.4	70.7	70.2	70.3	74.9	
Tot. Field Av. (%)*	17	**91.8**^{a}	**94.6**^{ab}	**102.0**^{bc}	**101.7**^{bc}	**101.4**^{bc}	**108.9**^{c}	<0.0029
95% conf.		+/-7.3	+/-5.4	+/-5.6	+/-6.2	+/-9.8	+/-8.7	

N = number of farmers, NoT = No Treatment, H_2O = hydropriming, Ea2.5 = *Eclipta alba* 2.5%

Variety Kapelga was used in all plots.

* Columns with same letters are not significantly different (Mann-Whitney paired test, p<0.05).

Participatory seed treatment of farm-saved and formally produced seeds

A one-year comparison was made between farm-saved seeds and seeds propagated by IN.E.R.A (Table 5). Experiments were carried out on fertilized fields in the same area (South-Eastern zone) as for experiments testing the effect of fertilizer above. Farmers were conducting seed treatment of the seeds and testing was conducted on the farmers own field. A substantial and similar increase of yield was observed for both formally produced and farm-saved seeds (+35% and +28%, respectively). Overall, the effect of seed treatment on all seeds considered as one group was significant (N=12, p<0.01). The finding indicates that the protocol for seed treatment is feasible and relevant to be tested by farmers in the South-Eastern region of Burkina Faso.

Table 5. *Eclipta alba* extract tested on formally propagated and farm-saved seeds (South-Eastern zone, 2016, fertilizer applied).

Seeds	N	Yield (kg/ha)		Yield (field averaged%)	
		NoT	Ea2.5	NoT	Ea2.5
Kouria	6	226	304	85.3	115
Farm-saved	6	255	341	87.7	112
Mean*	12	**240**	**323**	**86.5**[a]	**114**[b]
95% conf.		Na	Na	+/-7.1	+/-7.1

Treatment with 2.5% *E. alba* extract was tested on 6 fields. On each field both farm-saved seeds and formally propagated seeds of variety Kouria were tested.

N = number of fields, NoT = No Treatment, Ea = *Eclipta alba,* na = not applicable.

*Columns with same letters are not significantly different (t-test, p<0.01).

Perspectives

Since the use of *E. alba* extract in some locations can produce a substantial yield increase even without the use of fertilizer it represents in such areas an attractive first step in the "ladder approach" for intensifying agriculture as described for Western Africa by Aune and Bationo (2008). For the South-Eastern Burkina Faso, represented in the present study by villages Diapangou and Nakoaléongo, the technology of seed treatment using *E. alba* extract appears highly relevant: *E. alba* is commonly found in the region (Zida *et al.*, unpublished survey) and the small-scale, participatory trial using farm-saved seeds worked out successfully. A participatory approach eventually could be extended to include other measures such as micro-dosing of fertilizer and/or the use of soil formations (Zipellé/half-moon, Zaï, tied-ridges) to preserve soil and water, (Zougmoré *et al.*, 2003; Palé *et al.*, 2009; Aune *et al.*, 2017).

Conclusion

We have found for the first time a significant, yield increase in sorghum when applying *E. alba* extract to seeds of sorghum for growing on non-fertilized fields. A substantial, additional effect on yield was found when the treatment was combined with the use of fertilizer. The seed treatment technology using 2.5% plant extract appeared feasible for implementation in a participatory field trial in South-Eastern Burkina Faso testing both farm-saved seeds and formally propagated seeds.

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