# Effect of fungicidal formulations based on Cymbopogon citratus essential oil and two contrasted natural clays on seed health and seedling development of naturally pathogen-infested sorghum

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**ABSTRACT:** Sorghum is the most cultivated cereal in Burkina Faso. However, sorghum is produced extensively without respect for good agricultural and storage practices and the resulting grains are permanently infected by mold species. These mold species cause seeds rot, seedling damping-off and others diseases in field. They also secrete mycotoxins harmful to human health. The main method recommended for their control is seed treatment with chemicals. However, handling pesticides presents high risks of toxicity and ecotoxicity. The search for an alternative method made it possible to identify the essential oil of *Cymbopogon citratus* which has antifungal properties. This essential oil was then formulated by adsorption on clay in order to facilitate its use in the field. The objective of this study was to test the efficacy of the formulation made with two types of clay against *Phoma sorghina* and *Fusarium moniliforme*, the main species of molds encountered, and to evaluate the effect of the formulations. The grains thus coated were first analyzed to assess the presence of the target molds and then sown in pots containing sterilized fine sand to assess the germination and growth of the seedlings. The formulations significantly reduced grain infection rates from both fungi by more than 70%. The formulation with Sitiéna clay significantly improved germination, plant vigor and root length of both varieties. Field trials are necessary to confirm the efficacy of this formulation and initiate the popularization procedure.

**KEYWORDS:** *Cymbopogon citratus,* Essential oil, clay, *Fusarium moniliforme,* Sorghum.

# **1** INTRODUCTION

According to [1], there is global need to produce 70% more food by 2050 for an expected additional 2.3 billion people. It is therefore vital to undertake research towards increasing productivity of major food crops worldwide. Sorghum is a leading cereal in the arid and semi-arid regions of the world [2]. It is the major food crop used by rural populations within the semi-arid area in Africa, and in 2013, 25.7 million tons of grain sorghum was produced as compared to 23.5 and 8.8 million tons in America and Asia, respectively, making Africa the largest sorghum producer in the world [3].

A plant with such vast importance demands increases in productivity. This can be achieved by overcoming the several constraints including pests which are weakening sorghum production. Many fungal diseases are reported on sorghum in Burkina Faso and are mainly seed transmitted [4]. Seed-borne fungi of sorghum can cause damage on stored grains, lack of germination, mortality after germination and other anomalies in the field ([5], [6]. Therefore, effects of these micro-organisms affect the productivity of the plants. According to [7], pathogenic fungi alone cause nearly 20% reduction in the yield of major

food and cash crops. Fusarium moniliforme Sheldon and Phoma sorghina (Sacc.) are the major mold fungi frequently met on sorghum seed and other cereals grown in Burkina Faso [8], [4], [9], [10].

Seed treatment is one of the means of allowing the establishment of healthy crops [11]. This method uses many techniques among which, seed coating by chemical products is the main technique currently applied to control seed-borne fungi [12]. Widespread public concern for health and environmental effects of synthetic fungicides and the restriction of their use creates an opportunity for alternative products in particular reduced-risk pesticides [13], [14], [15]. The antifungal activity of many plant-derived products (Essential oil, aqueous extract, crude oil, leaf powder etc.) against a wide range of phytopathogens has frequently been documented [13], [16], [4], [17], [9], [18], [19], [11], [20], [21].

In previous work, we developed an efficient antifungal product based on essential oil of *Cymbopogon citratus* adsorbed in natural clay, against sorghum seed-borne fungi (Dossa et al., unpublished). This biofungicide has advantage of being safe, easy to apply and conserve. It could be an efficient alternative to synthetic products. However, the likely influence of clay types on the efficacy of this biofungicide is a main concern. Moreover, it is proved that some plant extracts could exhibit adverse influence on seedling emergence and vigor [22], [23]. Ref. [24] demonstrated that treatment with aqueous extract of sunflower, inhibited germination and growth of sorghum seed. Ref. [4] showed that Neem crude oil and *C. citratus* essential oil had inhibitory effect on sorghum seedlings growth rate.

The present work aimed to assess 1-the effect of seed treatment with biofungicide formulation based on *C. citratus* essential oil and natural clay on sorghum seed germination and seedling growth, 2-the influence of clay type on efficacy of the biofungicide.

# 2 MATERIAL AND METHODS

### 2.1 SORGHUM SAMPLES

The sorghum samples used included Kapelga and Framida obtained respectively from sorghum improvement program, INERA Farako-bâ and from the company "Neema Agricole du FASO" (NAFASO) in Burkina Faso. There were naturally infected by *P. sorghina* and *F. moniliforme* (Table 1).

Accession name	Variety	Location	Dericarn color	Infection rate		
			Pericarp color	P. sorghina	F. moniliforme	
Kapelga	Local	South/West	White	65%	11%	
Framida	Local	South/West	Red	28%	59%	

### Table 1. Sorghum seed samples collected in southwestern region of Burkina Faso

### 2.2 PREPARATION OF CLAY-ESSENTIAL OIL FORMULATIONS

Clay particles were ground into small mortar and sieved through a 230-mesh sieve to remove the larger non-clay fractions for obtaining clean clay powder. The powder obtained was sterilized and kept in closed vial. The essential oils of *C. citratus* were prepared by the Research Institute for Applied Sciences and Technology (IRSAT) of Burkina Faso. Extraction was performed by using stream distillation. The method used to prepare formulations was the same applied by [25]. Formulations were prepared by using the following ratio:

 $\frac{m_{EO}}{m_{Clay}}$  = 0.1 with mEO: mass of essential oil; mClay: mass of clay powder.

To prepare 10 g of formulation, 10 g of clay powder were transferred in a 100 mL flask. 1,16 mL of *C. citratus* essential oils (diluted in 10 mL of acetone) was added. After 5 min of manual shaking, the mixture was placed in a water bath thermostated at 30°C for 90 min to complete the evaporation of acetone. The aromatized powders obtained were kept in vials and tightly closed.

# 2.3 SEED TREATMENT

Seeds were coated with single clays, aromatized powders and the synthetic fungicide applied in the following dose: (i) seeds treated with synthetic fungicide Calthio C. composed of 25% chlorpyrifos-ethyl plus 25% Thiram WS (applied at 20 g for 5 kg of seeds); (ii) seeds treated respectively with clay 'Kôrô' and clay 'Sitiéna' applied at 800 g for 5 kg of seeds; (iii) seeds coated with clay "Kôrô"-essential oil formulation of *C. citratus* applied at 800 g for 5 kg of seeds; (iv) seeds coated with clay "Sitiéna"-essential oil formulation of *C. citratus* applied at 800 g for 5 kg of seeds.

After treatment, the flasks were covered with Parafilm<sup>®</sup>, manually shaken for 5 min and kept in the laboratory between 28°C and 30°C for 24 h.

# 2.4 TREATED SEED HEALTH TESTING

To assess the health quality of seeds after treatment, the "Blotter method" as described by [26] was used. 400 seeds per sample were tested in 16 replications of 25 seeds following the international rules for seed testing (Mathur and Kondal, 2003). For each replication, 25 seeds were placed equidistant on three layers of moistened blotter papers in a sterile Petri dish. They were incubated at 25°C under a light-dark cycle (12/12 h) for 7 days. The seeds were then individually observed under a stereomicroscope at 16x and 25x magnifications in order to detect the presence of fungal colonies and identify the species based on morphological characteristics.

For each fungal species found, the number of infected seeds was evaluated by counting and the infection rates (If (%)) by each fungus were calculated using the following formula:

IF (%) = Number of seeds infected by a fungus/ Total number of analyzed seeds \* 100

The reduction in infection rate of treated seeds compared to untreated seeds (absolute control) was calculated as follows:

# Red (%) = [(IFnt –IFt) / IFnt] x 100

Where: IFnt: Infection rate of untreated seeds; IFt: Infection rate of treated seeds.

# 2.5 TESTING EFFECT OF AROMATIZED POWDERS ON SEED GERMINATION AND SEEDLING GROWTH

One hundred seeds per treatment were used for the seed germination test. The seedling substrate used was white sand from the sandpit of 'Kôrô village'. The sand was sterilized and allowed to cool, then put in plastic pots (24 cm diameter x 9 cm depth). 25 seeds were sown per pot arranged in circles: 15 seed holes in the outer ring, 9 in the middle ring and one in the center. One seed was planted in the holes of 1 cm deep and 0.9 cm in diameter. The pots were watered 24 h after planting and subsequently as needed. This experiment was carried out in the open-air conditions, at temperatures ranging between 28°C in the evening to 39°C at midday. After 13 days, seedling emergence was recorded. 21 days after sowing, to find out the seedling vigor, 4 well developed plants were taken from each pot per treatment. The root length (cm) and shoot length (cm) were measured. In addition, total biomass (cg) was recorded. Vigor index was calculated according to the formula of [27].

# $VI = SG \times (SL + RL)$

Where: VI – vigor index, SG – seed germination (%), SL – mean shoot length (cm), RL – mean radicle length (cm), (SL + RL) – seedling length.

The experimental design was a completely randomize block design with 6 treatments as follows: Ta (untreated seeds); Tf (seeds treated with synthetic fungicide calthio C. composed of 25% chlorpyrifos-ethyl plus 25% Thirame WS; C1 and C2 (seeds treated respectively with clay 'Kôrô' and clay 'Sitiéna'; Cy1 (clay "Kôrô"-essential oil formulation of *C. citratus*; Cy2 (clay "Sitiéna"-essential oil formulation of *C. citratus*. Each treatment was repeated 4 times.

# 2.6 STATISTICS

Data are initially recorded in Microsoft Excel 2007<sup>®</sup> and were subjected to analysis of variance (ANOVA) using R2.15.2 software with packages "agricolae" [28] and "GrapheR" [29]. When the variance analysis detects significant differences among the treatments, the means calculated are classified using the multiple comparison of Tukey at 5% level.

# 3 RESULTS

### 3.1 EFFECT OF SEED TREATMENT ON THE INCIDENCE OF *P. SORGHINA* AND *F. MONILIFORME*

The statistical analysis revealed a highly significant difference between the treatments. Results showed that seed treatment with single clay powders didn't reduce fungi incidence compared to the untreated seeds of Kapelga and Framida (Table 2).

Seed treatment with both clay powders aromatized with *C. citratus* essential oil applied at 800 g for 5 kg of seeds reduced considerably the infection by *P. sorghina* (88 and 94 % respectively for Cy1 and Cy2 in Kapelga; 93 and 71 % respectively for Cy1 and Cy2 in Framida). Both aromatized powders of the two clay types had the same effect in controlling the fungus as the synthetic fungicide in the two sorghum varieties.

Concerning *F. moniliforme*, seed treatment with the aromatized powders didn't exhibit significant effect in controlling the fungus in Kapelga compared to the untreated seeds. However, an adverse effect was observed against this fungus in Framida seeds treated with the aromatized powders.

In general, the efficacy of the aromatized powders didn't vary according to the color of the variety.

# Table 2. Infection rate (%) and (percentage reduction in infection rate (%) of sorghum seeds by P. sorghina and F. moniliforme depending on the treatments

Treatments	P. sorghina		F. moniliforme		
Treatments	Kapelga	Framida	Kapelga	Framida	
Та	16.3ª	7.0 <sup>a</sup>	2.8 <sup>ab</sup>	14.8ª	
Tf	1.5 <sup>b</sup> (90.7)	0.3 <sup>b</sup> (95.7)	1.9 <sup>b</sup> (32.1)	7.3 <sup>b</sup> (50,7)	
C1	17.9ª (-9.8)	5.0ª (28.6)	3.0 <sup>ab</sup> (-7,1)	15.6ª (-5,4)	
C2	15.0ª (7.9)	5.3° (24.3)	4.9 <sup>a</sup> (-75.0)	15.9ª (-7,4)	
Cy1	2.0 <sup>b</sup> (87.7)	0.5 <sup>b</sup> (92.9)	1.6 <sup>b</sup> (42,9)	3.9 <sup>b</sup> (73.6)	
Cy2	1.0 <sup>b</sup> (93.9)	2.0 <sup>b</sup> (71,4)	4.0 <sup>ab</sup> (-42.9)	7.9 <sup>b</sup> (46.6)	

Ta (untreated seeds); Tf (seeds treated with synthetic fungicide calthio C. (20 g for 5 kg of seeds)); C1 and C2 (seeds treated respectively with clay 'Kôrô' and clay 'Sitiéna' applied at 800 g for 5 kg of seeds) Cy1 (clay 'Kôrô'-essential oil formulation of *C. citratus* applied at 800 g for 5 kg of seeds) and Cy2 (clay 'Sitiéna'-essential oil formulation of *C. citratus* applied at 800 g for 5 kg of seeds). Values are means. The numbers followed by the same letter in the same column are not significantly different at the level 5% according to the multiple classification of Tukey.

# 3.2 EFFECT OF TREATMENTS ON SORGHUM SEED GERMINATION

The red variety "Framida" showed seed germination rate higher than the white variety. Compared to the untreated seeds of both varieties, seeds treatment with the aromatized powders didn't inhibit seed germination. On the contrary, "Sitiéna" clay formulation increased seed germination of 37 % and 17 % respectively in Kapelga and Framida (Fig. 1).

"Sitiéna" clay formulation was the most potent as compared to the synthetic fungicide in increasing seed germination of the two varieties.



 Fig. 1.
 Seed germination, in sorghum varieties treated with different aromatized powders. A-Variety Kapelga; B-Variety Framida.

 Means followed by same letters above histogram bars are not significantly different (p < 0.05).</td>

# 3.3 EFFECT OF TREATMENTS ON SORGHUM SEEDLING GROWTH

Statistical analysis revealed significant difference between treatments. Seedlings obtained from untreated seeds of Kapelga showed the lowest shoot length as well as plants from seeds treated with "Kôrô" clay formulation. "Sitiéna" clay formulation increased seedling growth in Kapelga variety more than "Kôrô" clay formulation. It was as potent as the synthetic fungicide. However, in Framida variety, Seed treatments didn't result in improve of seedlings height. On the contrary, "Kôrô" clay formulation showed slight inhibition effect on height of seedlings from treated seeds compared to seedlings from untreated seeds.



 Fig. 2.
 Shoot length in sorghum varieties treated with different aromatized powders. A-Variety Kapelga; B-Variety Framida.

 Significant differences (p < 0.05) between means were indicated by different letters above histogram bars.</th>

# 3.4 EFFECT OF SEED TREATMENT ON SORGHUM SEEDLINGS BIOMASS, ROOT LENGTH AND VIGOR

The results obtained for the weight of the 4 well developed plants of the white variety Kapelga indicated that "Kôrô" clay formulation improved the total biomass of sorghum plants more than the others treatments (Table 4). However, it exhibited depressive effect on Framida seedlings total biomass contrary to the others treatments. Framida variety showed much higher total biomass than Kapelga variety. "Sitiéna" clay formulation and the synthetic fungicide increased highly root length of seedlings of both varieties as compared to seedlings from "Kôrô" clay formulation treatment and untreated seeds.

Statistical analysis of Vigour index showed highly significant difference between the different treatments. Framida seedlings are more vigor than Kapelga seedlings (579.7 vs 289.3) which is naturally more infected by P. sorghina (65 % vs 28 %). In general, "Sitiéna" clay formulation improved considerably seedlings vigour for both varieties. The fungicide treatment also resulted in increase of seedlings vigor of Kapelga but "Koro" clay formulation seemed to have no improving effect on seedlings vigour as compared to seedlings from untreated seeds.

Treatments	Kapelga			Framida		
	Biomass (cg)	Root length (cm)	Vigor index	Biomass (cg)	Root length (cm)	Vigor index
Та	1.21 <sup>b</sup>	9 <sup>b</sup>	289.3 <sup>b</sup>	1.88ª	13.8 <sup>b</sup>	579.7 <sup>ab</sup>
Tf	1.78 <sup>ab</sup>	14.9ª	555.4ª	1.85ª	18.8ª	593.4 <sup>ab</sup>
Cy1	1.92ª	8.8 <sup>b</sup>	296.7 <sup>b</sup>	1.43 <sup>b</sup>	10.5 <sup>b</sup>	403.7 <sup>b</sup>
Cy2	1.18 <sup>b</sup>	15.8ª	551.2ª	1.98ª	17.7ª	723.2ª

### Table 3. Effect of seed treatment on sorghum seedlings biomass, root length and vigor

**Ta** (untreated seeds); Tf (seeds treated with synthetic fungicide calthio C. (20 g for 5 kg of seeds)); **Cy1** (clay 'Kôrô'-essential oil formulation of *C. citratus* applied at 800 g for 5 kg of seeds) and **Cy2** (clay 'Sitiéna'-essential oil formulation of *C. citratus* applied at 800 g for 5 kg of seeds). Values are means. The numbers followed by the same letter in the same column are not significantly different at the level 5% according to the multiple classification of Tukey.

# 4 DISCUSSION

Sorghum is poised to play a key role in expanding agricultural development and food security in developing countries. However, sorghum seeds are strongly contaminated by pathogenic seed-borne fungi which affect its productivity. In Burkina Faso, these fungi can reduce seed germination by 4 to 12 % and their transmission rate to the plants varies from 63 to 100 %, causing the death from 15 to 65 % of them [9]. The present study is a contribution in research of environmentally safe ways of pathogenic fungi management in aim of sorghum productivity improvement through use of a formulation based on natural clay and essential oil.

# 4.1 EFFECT OF SINGLE CLAYS AND CLAY-ESSENTIAL OIL FORMULATIONS APPLIED AS BIO-FUNGICIDE

Our findings showed that both single natural clays applied as seed treatment against seed-borne fungi didn't exhibit antifungal activity. However, formulations based on essential oil of C. citratus adsorbed on the different clay powders considerably reduced the incidence of *Phoma sorghina* and *Fusarium moniliforme* in both varieties. Clay powder appears thereby, to be a simple carrier of the active molecules of the essential oil and doesn't affect fungi incidence. These results contrasted with previous reports of [30] and later on, [31] who demonstrated that the foliar application of clay can reduce the severity of powdery mildew (caused by pathogenic fungi) on different crops. Indeed, clay is thought to prevent plant diseases by acting as a particle film barrier preventing disease inoculum from directly contacting plant tissue [32], but any study in our knowledge have reported the fungicidal properties of natural clay. We suggest further studies in this way with broad range of pathogenic fungi and natural clays applied as bio-fungicide.

In previous work (Dossa et al., unpublished), we demonstrated the potential of the formulation based on natural clay and plant essential oil against wide range of pathogenic seed-borne fungi. Clay-essential oil formulation is more stable, easy to apply, to conserve and efficient in fighting seed-borne fungi than essential oil use single. By reducing pathogenic fungi in sorghum seeds, the bio-fungicide offers healthy conditions for seed germination.

# 4.2 EFFECT OF CLAY TYPE ON EFFICACY OF THE FORMULATION IN INCREASING SEEDLINGS DEVELOPMENT AND VIGOR

Good seed must have a high germination rate and be free of seed-borne pathogens [9]. In our study, both clays fungicidal formulations have an adverse effect on seed-borne fungi but seed germination test clearly showed that "Sitiéna" clay formulation better increased germination than all the others treatments. Moreover, seedlings shoots and roots were highly improved for both varieties with "Sitiéna" clay formulation and the best vigor indexes were recorded in both varieties with "Sitiéna" clay formulation. This result may due to the characteristic of "Sitiéna" clay.

# 4.3 ARE PHOMA SORGHINA AND FUSARIUM MONILIFORME PATHOGENIC FUNGI ASSOCIATED TO SEEDLING STAGE?

The white variety Kapelga is much more infected by P. sorghina than the variety Framida which is contrarily, the most infected by *F. moniliforme*. However, Framida seeds better germinated and its seedlings exhibited the best Vigor index despite the high incidence of *F. moniliforme*. These results showed that *F. moniliforme* doesn't affect negatively sorghum crop in seedling stage. F. moniliforme is known to be sorghum grain mold fungus [33], [34], [4] but could be a beneficial fungus for sorghum in seedling stage in view of the good performance of seedlings from untreated seeds of Framida. These findings are in agreement with results of [10] who found that *F. moniliforme* was much more encountered in performing plants of sorghum compared to non-performing plants.

On the other hand, the weak performance of Kapelga could be explained by the high presence of *P. sorghina* which seems to be a pathogenic fungus associated to earlier stage of development in sorghum. Many authors as [35] and [36] reported that P. sorghina causes mortality before and after germination of sorghum. In our study, treatment of Kapelga seeds with "Sitiéna" clay formulation eliminated most of pathogenic fungi including *P. sorghina* and thus promoted a higher germination rate compared to untreated seeds of Kapelga. Conclusion.

# 5 CONCLUSION

This study, which is part of the search for an alternative solution to the chemical treatment of sorghum seeds against mold, has made it possible to obtain information which opens up interesting perspectives.

The essential oil of *C. citratus* adsorbed on clay powder and used as a seed treatment product, indeed, made it possible to obtain results identical to those of the synthetic chemical fungicide. This indicates that this formulation can be an alternative to the use of chemical seed treatment products.

Clay and essential oil of C. citratus can be obtained at a lower cost by producers because C. citratus is cultivable and many clay deposits exist in many parts of the country. The adoption of this technology cannot therefore be hampered by the cost of formulation.

Field trials are needed to assess the efficacy and cost-effectiveness of the formulation on a large scale.

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