**EPH - International Journal of Agriculture and Environmental Research**

ISSN (Online): 2208-2158
Volume 07 Issue 02 December 2021

DOI: https://doi.org/10.53555/eijaer.v5i1.58

# FUNGICIDAL FORMULATION BASED ON ESSENTIAL OILS OF *CYMBOPOGON CITRATUS*, *LIPPIA MULTIFLORA* AND NATURAL CLAY IN CONTROLLING SORGHUM SEED-BORNE FUNGI

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## Abstract:-

*The fungicidal properties of formulation based on essential oils of Cymbopogon citratus, Lippia multiflora and swelling clayey powder containing montmorillonite (26 wt.%), iolites (21 wt.%), kaolinite (7 wt.%), orthoclase (20 wt.%), quartz (15 wt.%), goethite (2 wt.%) and hematite (4 wt.%) with specific surface area of 34.68 $m^2/g$ have been tested against sorghum seed-borne fungi, on two varieties (Framida and Kapelga). Aromatized powders were applied at 100 g and 800 g for 5 kg of seeds and the infection levels were evaluated after 7 days of incubation compared with untreated seeds and seeds treated with synthetic fungicide. The aromatized powders exhibited moderate to strong antifungal activity against the fungi Fusarium moniliforme and Phoma sorghina in both varieties. The mass formulation of 800 g for 5 kg of seeds showed the best antifungal activity. Clay-C. citratus oil formulation was the most potent as compared to the synthetic fungicide in the two varieties. It reduced P. sorghina infection by 87.50% and 92.85% in Framida and Kapelga respectively. F. moniliforme infection was reduced by 40.74% and 74.15% respectively in Kapelga and Framida. The findings suggest that formulation based on essential oils adsorbed on swelling clayey material can be considered as alternative to synthetic fungicide for use in controlling sorghum seedborne fungi.*

**Key-words:** - *Essential oils, swelling clayey material, seed-borne fungi, seed treatment, sorghum*

## INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is among the most important grain crops cultivated in the world. It occupies the fifth position after maize, rice, wheat and barley [1] (Sher *et al.*, 2013). It is the second most produced grain in Africa [2].

In Burkina Faso, sorghum and pearl millet make the staple food for 80% of the population [3]. However, its production is challenged by several constraints including pests. Many fungal diseases are reported on sorghum and are mainly transmitted by seed [4]. The mould fungus such as *Fusarium moniliforme* Sheldon is the most common fungus isolated from sorghum plants in Burkina Faso [5]. It causes seed and seedling root rot and is involved in the head mould complex [6]. In addition, *Phoma sorghina* (Sacc.) Boerema, Dorebosch and van Kesteren was also found at high incidence on seed samples from Burkina Faso and represents a major threat for sorghum plants [7]. This fungus can affect seed germination and causes mortality after germination.

Synthetic fungicides for seed treatment are expensive for subsistence farmers and they may pose potential risks owing to the lack of adequate technical knowledge related to their safe use. They have a proven toxicity to humans [8] and environment [9, 10]. Therefore, alternative methods with low adverse effects and less persistence on the environment are needed. The pesticidal properties of several local aromatic plants specifically essential oils extracted were tested with satisfactory results on several crops [7, 11, 12, 13, 14, 15, 16]. In Burkina Faso, [2]Bonzi et al. (2013) showed that essential oils of *Cymbopogon citratus* and *Lippia multiflora* significantly reduced sorghum seed infection by *P. sorghina* and can improve seedlings growth.

It emerges that essential oils are effective for seed treatment against fungi, but the major concern about their use remains their conservation because of their high volatility and biodegradability [17, 18].

The challenge is to develop an antifungal product easy to handle with an improved persistence based on active essential oils. [12, 19, 20] Kéita *et al.* (2000; 2001); Nguemtchouin et al. (2009) showed that essential oils can be fixed to a solid support such as clay and used it as insecticide carrier against pests. The studies conducted in this way only focused on insecticidal formulations based on natural or modified clays from Cameroon and essential oils [21, 22]. The clayey materials from Burkina Faso at our knowledge have been not tested. It is very known that the mineralogical composition and the specific surface area of clayey material is very determinant for the essentials oils adsorption.

The aim of this investigation was to determine, the fungicidal activities of powdered formulation based on essential oils of *C. citratus* (D.C.) Stapf. and *L. multiflora* Moldenke and natural clayey material from Burkina Faso against sorghum seed-borne infection by *F. moniliforme* and *P. sorghina* (Sacc.).

## Materials and methods

### Sorghum seed samples

Two varieties of sorghum naturally infected by *F. moniliforme* and *P. sorghina* were used. It included Kapelga with white pericarp and Framida with red pericarp obtained from sorghum improvement program, INERA Farakobâ and from the company “Neema Agricole du FASO” (NAFASO) in Burkina Faso.

### Preparation of the clay powder

Dried clay sample was collected in “Kôrô village” located in west of Burkina Faso (10°16'60" N and 03°01'00" W). 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.

### Clay Chemical and mineralogical characterizations

X-ray diffraction (XRD) and Differential Thermal Analyses (DTA) were used to study the mineralogical composition of the raw materials. The X-ray diffraction used apparatus was a Brüker 5000 AXS X-ray Diffractometer equipped with a monochromator using a Cu Kα radiation (□ = 1.54 Å). Chemical analyses were performed by ICP (Iris Plasma Spectrometer). The loss on ignition is evaluated by sample calcination until 1000°C during 3 h.

DTA were carried out on a crushed sample of clay heated up to 1000 °C at a constant rate of 10 °C/min using a Netzsch SATA 449 F3 Jupiter apparatus. To access to the specific surface area of the clay material, BET methods was used with the pre-treatment of the sample. This one was heated at 200°C during 24 h in order to eliminate all adsorbed gaz. Micromeritics TriStar II piloted with TriStar II 3020 apparatus was used. The mineralogical composition of the sample was obtained by using the results of X-ray diffraction and the chemical analyses. The following relation was used to calculate the amount T (a) in oxide (wt. %) of chemical element « *a* » :

$T(a) = \sum M_i P_i(a)$ where, $M_i$ is: amount (in wt.%) in mineral *i* in the material under study containing the element *i* ; $P_i$ *(a)* is: proportion of element *a* in the mineral *i*.

### Preparation of clay-essential oil formulation

The essential oils of *C. citratus* and *L. multiflora* were prepared by the Research Institute for Applied Sciences and Technology (IRSAT) of Burkina Faso. Extraction was performed by using steam distillation.

The method used to prepare formulations was the same applied by Nguemtchouin et al. (2010). Formulation was prepared by using the following ratio:

$\frac{mEO}{mClay}$ = 0.1 where mEO is: mass of essential oil and mClay is: mass of clay powder.

To prepare 10 g of formulation, 10 g of clay powder were transferred in a 100 mL flask. 1.14 mL and 1.16 mL respectively of *L. multiflora* and *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 powder obtained was kept in vials and tightly closed.

**Experimental design**

The experimental design was a completely randomized 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 (applied at 20 g for 5 kg of seeds)); Cy100, Cy800 (clay-essential oil formulation of *C. citratus* applied at 100 g and 800 g for 5 kg of seeds respectively) and Li100, Li800 (clay-essential oil formulation of *L. multiflora* applied at 100 g and 800 g for 5 kg of seeds respectively). Each treatment was repeated 4 times.

N.B. We tested several mass formulations 20 g, 50 g, 100 g and 800 g for 5 kg of seeds. But we just retained 100 g and 800 g because they revealed a clear effect on the fungi incidence.

**Seeds treatment, plating and incubation**

Seeds were coated with the aromatized powders and the synthetic fungicide applied in dose described above. After treatment, the flasks were covered with Parafilm®, manually shaken for 5 min and kept in laboratory between 28°C and 30°C for 24 h.

25 seeds were plated in each Petri dish containing three wet filter papers. After plating, the Petri dishes were incubated under 12 h alternating cycles of near ultraviolet (NUV) and darkness at 22°C for 7 days according to [23]Mathur and Kongsdal, (2003).

**Evaluation and statistical analysis**

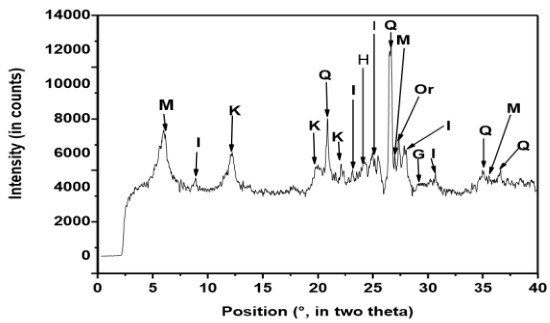
After incubation, the seeds were examined under a compound microscope to identify fungi developing on the different seeds and results were recorded on the seed health report. Data were recorded in Microsoft Excel and analyzed using one-way analysis of variance (ANOVA) with Minitab 16 Software. The partitioning of the means was made with Tukey test at 5% probability level.

**Results**

**Mineralogical and chemical characterizations of the clay material:**

The specific surface area of the studied sample by BET method was 34.68 $m^2/g$. The powder X-ray diffraction pattern of the clay material sample is shown in Figure 1. The X-ray diffraction showed that the sample was essentially composed of quartz ($SiO_2$), kaolinite (Al2Si2O5 (OH)4), illite (K $Al_2$ ($AlSi_3$) $O_{10}$ $(OH)_2$), montmorillonite **(**(Al1,67Mg0,33)Si4O10(OH)2Na0,33**),** goethite (FeO(OH)), hematite ($Fe_2O_3$) and orthoclase ($KAlSi_3O_8$). To complete the mineralogical characterization and identify the amorphous compounds, the sample was subjected to differential thermal analysis (DTA). Results are presented in Figure 2. The interpretations of the thermogram were supported by the works of [24]Millogo *et al.* (2011). The endothermal peak around 100°C was attributable to loss of the hygroscopic water. The endothermal peaks around 265, 500 and 574°C characterized the dehydroxylation of goethite, dehydroxylation of clay minerals (kaolinite, illite and montmorillonite) and the allotropic transformation of quartz (α to β) respectively. The exothermal effects around 310 and 900°C were due to the decomposition of organic matter and the recrystallization phenomenon likely to the spinel formation respectively. The broad endothermal effect around 100°C confirmed that the sample is rich in swelling clay minerals.

The chemical composition showed a high amount of silica and alumina, an appreciable quantity of iron oxide and feeble amount of magnesium and potassium oxides (Table 1). Based on the chemical composition, minerals identified by X-ray diffraction and the works published by [25]Millogo *et al.* (2014), the sample was composed of kaolinite (7 wt.%), quartz (18 wt.%), illite (21 wt.%), goethite (2 wt.%), montmorillonite (26 wt.%), hematite (4 wt.%) and orthoclase (20 wt.%).




**K: kaolinite, I: illite , M : montmorillonite , Q: quartz , G: goethite , H: hematite , Or: orthoclase**

**Figure 1. X-ray diffraction pattern of the sample**

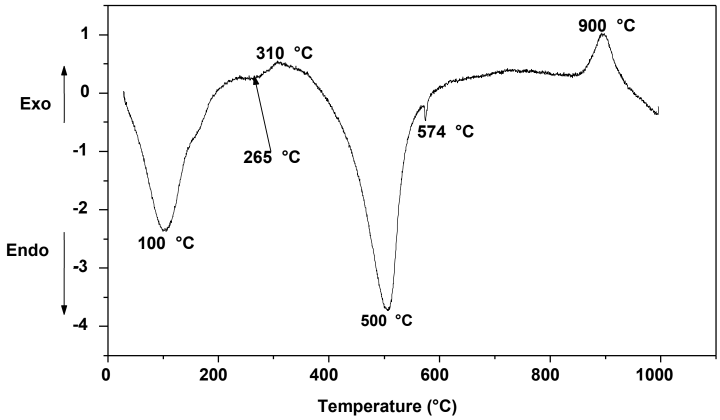



**Figure 2. Differential thermal analysis of the studied clay material**

**Table 1. Chemical composition of the raw material**

| Oxides | $SiO_2$ | $Al_2O_3$ | $Fe_2O_3$ | MgO | CaO | $Na_2O$ | $K_2O$ | $TiO_2$ | LOI* | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Wt.% | 57.2 | 20.3 | 6.53 | 1.12 | 0.99 | 1.49 | 2.91 | 0.71 | 5.26 | 96.51 |

* Loss on ignition at 1000

**Effect of seed treatment on the incidence of *P. sorghina*:**
The statistical analysis revealed a highly significant difference between the treatments. Treatments with the powders of *L. multiflora* and *C. citratus* applied at 100 g to 5 kg of seeds reduced slightly the infection of *P. sorghina* compared to the untreated seeds of both Kapelga and Framida. But the powders applied at 800 g to 5 kg of seeds, showed a better efficacy in controlling the fungus. It was envisaged from results that the powder aromatized with *C. citratus* essential oil applied at 800 g to 5 kg of seeds was the most effective and produced the same effect as the synthetic fungicide. It was very effective on red as compared to white sorghum varieties (Table 2).

**Table 2. Effect of seed treatment on the incidence of *P. sorghina* in sorghum seed.**

| Treatments | Kapelga | Framida |
|---|---|---|
| Ta | 16.3 [a] | 7.0 [b] |
| Tf | 1.5 [d] | 0.3 [c] |
| Cy100 | 13.5 [b] | 8.0 [b] |
| Li100 | 12.0 [b] | 13.1 [a] |
| Cy800 | 2.0 [d] | 0.5 [c] |
| Li800 | 5.0 [c] | 4.9 [b] |

Ta (untreated seeds); Tf (seeds treated with synthetic fungicide calthio C. (20 g for 5 kg of seeds)); Cy100, Cy800 (clay-essential oil formulation of *C. citratus* applied at 100 g and 800 g for 5 kg of seeds) and Li100, Li800 (clay-essential oil formulation of *L. multiflora* applied at 100 g and 800 g for 5 kg of seeds). Values are means of 4 replications. 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.

**Effect of seed treatment on the incidence of *F. moniliforme*:**
Two sorghum varieties naturally infected at different rate by *Fusarium moniliforme* were treated to evaluate effectiveness of the aromatized powders in controlling the fungus. The red variety Framida was more infected than the white variety (14.7 vs 2.7) as shown in Table 3. The recorded data showed a high level of significant difference ($P < 0.05$) between the six treatments. Both aromatized powders applied at 800 g for 5 kg of seeds and the fungicide calthio C. reduced significantly the infection of *F. moniliforme* compared to the untreated seed. Efficacy of both aromatized powders in controlling the fungus was higher than the fungicide calthio C.

**Table 3. Effect of seed treatment on the incidence of *F. moniliforme* in sorghum seed.**

| Treatments | Kapelga | Framida |
|---|---|---|
| Ta | 2.7 [b] | 14.7 [a] |
| Tf | 1.8 [bc] | 7.2 [c] |
| Cy100 | 0.6 [bc] | 11.1 [b] |
| Li100 | 5.3 [a] | 7.3 [c] |
| Cy800 | 1.6 [bc] | 3.8 [cd] |
| Li800 | 0.3 [c] | 2.5 [d] |

Ta (untreated seeds); Tf (seeds treated with synthetic fungicide calthio C. (20 g for 5 kg of seeds)); Cy100, Cy800 (clay-essential oil formulation of *C. citratus* applied at 100 g and 800 g for 5 kg of seeds) and Li100, Li800 (clay-essential oil formulation of *L. multiflora* applied at 100 g and 800 g for 5 kg of seeds). Values are means of 4 replications. 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.

**Effect of seed treatment on the incidence of whole sorghum seed-borne fungi:**
Apart from the two targeted fungi namely *P. sorghina* and *F. moniliforme*, the effect of formulations on whole observed fungi was assessed. Of all samples observed, untreated seeds of Kapelga and Framida showed the highest numbers of fungi with a wide spectrum of pathogenic fungi such as *Curvularia spp., Bipolaris spp., Fusarium spp., Colletotrichum graminicola, Alternaria spp., Cladosprium sphaerospermum, Phaeotrichoconis crotalarieae, Rhisopus spp., Aspergillus flavus*. Seeds treated with the aromatized powders applied at 800 g for 5 kg of seeds reduced the number of whole observed fungi in both varieties (Figure 3). Moreover, powder containing *C. citratus* oil better reduced whole fungi compared to the *L. multiflora*based powder.

Moreover, the infection rates of some important pathogenic fungi such as *Curvularia spp.* (Cu spp.), *Alternaria spp*. (All spp.), *Bipolaris bicolor* (Bb) and *Cladosprium sphaerospermum* (Cs), were considerably reduced by *C. citatus*-based powder applied at 800 g for 5 kg of seed similar to the synthetic fungicide (Table 4, photo 1).

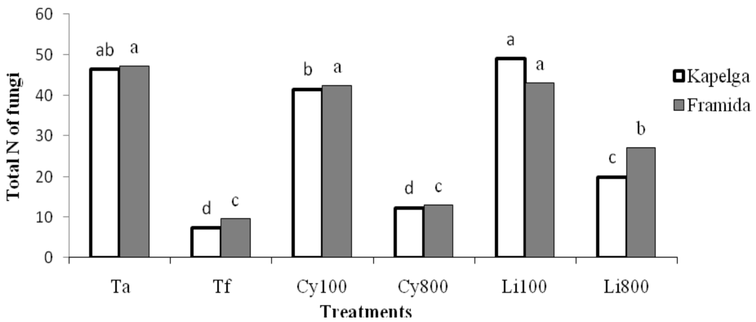



**Figure 3. Effect of seed treatment on whole fungi observed in sorghum seeds.**

**Table 4. Effect of seed treatment on the infection rate (%) of some other important pathogenic fungi in sorghum seed**

| Fungi | Infection rate (%)/Kapelga | | | | Infection rate (%)/Framida | | | |
|---|---|---|---|---|---|---|---|---|
| | Ta | Tf | Cy800 | Li800 | Ta | Tf | Cy800 | Li800 |
| Cu spp. | 46 | 14 | 22.5 | 31.5 | 16.5 | 6 | 2 | 17 |
| All spp. | 17 | 3 | 4.5 | 11.5 | 3 | 0.5 | 0 | 3.5 |
| Bb | 3.5 | 0.5 | 1.5 | 1 | 0 | 0.5 | 0 | 0.5 |
| Cs | 51 | 0 | 1.5 | 10.5 | 87 | 0.5 | 8.5 | 54 |

Ta (untreated seeds); Tf (seeds treated with synthetic fungicide calthio C. (20 g for 5 kg of seeds)); Cy800 (clay-essential oil formulation of *C. citratus* applied at 800 g for 5 kg of seeds) and Li800 (clay-essential oil formulation of *L. multiflora* applied at 800 g for 5 kg of seeds). Cu spp. (*Curvularia spp.*), All spp. (*Alternaria spp*.), Bb (*Bipolaris bicolor*) and Cs (Cladosprium sphaerospermum)

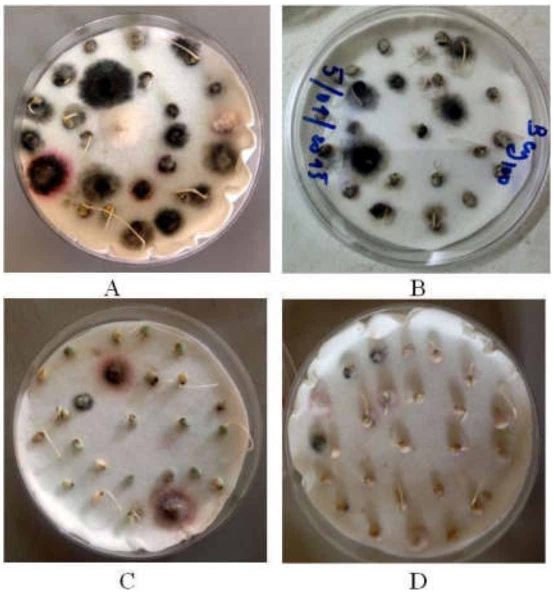



**Photo 1.** Effect of seed treatment on reduction of seed-borne infection of fungi associated with sorghum seeds (Kapelga). A- Huge growth of seed-borne fungi (Control). B- Seed treated with Formulation based on *C. citratus* essential oil (100 g for 5 kg of seeds) showed low effect in controlling seed-borne fungal infection. C- Almost complete control of seedborne fungal infection (Calthio C.). D- Seed treated with Formulation based on *C. citratus* essential oil (800 g for 5 kg of seeds) showed highest control of seed-borne fungal infection.

**Discussion**

Seed-borne fungi of sorghum can cause damage on stored grains, lack of germination, mortality after germination and other anomalies in the field [26](Islam *et al*., 2009). Therefore, effects of these micro-organisms affect the productivity of the plants. According to [27]Agrios, (2000), pathogenic fungi alone cause nearly 20% reductions in the yield of major food and cash crops. Seed treatment is one of the approaches of obtaining healthy crops. In this study, we developed and tested a natural fungicide based on essential oils of *C. citratus* and *L. multiflora* and clay as alternative to synthetic fungicide which are expensive and harmful to human and environment [9].

Taking into account the result of the specific surface area, it could be concluded that the sample is rich in smectite clay minerals and it is suitable for essential oils adsorption. The sample was rich in swelling clay mineral such as montmorillonite as was shown the specific surface area value and this is very interesting because this clay mineral could adsorb essential oils trough its interlamelar space.

The aromatized powders obtained by adsorbing the essential oils in the clay, exhibited moderate to strong antifungal activity against *Phoma sorghina* and *Fusarium moniliforme* in Kapelga and Framida. The best antifungal activities were observed with the mass formulation of 800 g for 5 kg of seeds for both aromatized powders. In general, powder aromatized with *Cymbopogon citratus* essential oil appears to be the most potent with a same effect as the synthetic fungicide in the two varieties. These findings are in agreement with the results obtained by [2]Bonzi *et al.* 2013 who obtained the best antifungal activity using essential oil of *C. citratus* in controlling *P. sorghina* infection in sorghum seed compared to essential oil of *L. multiflora*. Same results were also found by [3]Somda *et al*. 2007 using essential oil of *C. citratus* against *C. graminicola*, *P. sorghina* and *F. moniliforme*.

*L. multiflora*-based powder is particularly more effective against *Fusarium moniliforme*. According to[28] Bassolé *et al.* (2011), *C. citratus* essential oil is rich in Gera-nial (48.1%), neral (34.6%) and myrcene (11.0%) whereas *L. multiflora* essential oil is rich in p-cymene, thymol, β-caryophyllene, carvacrol and carvone [29]. The difference of efficiency observed between both aromatized powders may be related to the difference of essential oils chemical composition.

[2]Bonzi *et al*. 2013 also found that *L. multiflora* essential oil failed in reducing *P. sorghina* infection in red seed compared to white seed. This was explained by the presence of anthocyanic and tannin substances that decrease the antifungal substance absorption. In this study, powder aromatized with essential oil of *L. multiflora* applied at 800 g for 5 kg of seeds didn't have effect against *P. sorghina* but displayed the high fungicidal activity against *F. moniliforme* in the red seed. Further investigations will be needed in order to clearly demonstrate the effect of sorghum seed color on efficacy of natural fungicidal products.

[4]Zida *et al.* 2014 isolated a total of 39 fungal species including many pathogenic species from sorghum plants in Burkina Faso. Therefore, development of fungicide with effect on a large spectrum of fungi is another key issue. Clay powder aromatized with *C. citratus* essential oil applied at 800 g for 5 kg of seeds reduced the infection of most observed pathogenic fungi. Furthermore, [30]Yago *et al*. 2011 found that 4 out of the 5 dominant sorghum seed-borne fungi were observed as dominant fungi in foxtail millet in South Korean.

This biofungicide could then find its application on other important crops.
*C. citratus* essential oil and clay could be an efficient alternative to synthetic products in controlling sorghum seed-borne fungi. The advantage of using clay as a carrier for essential oils is that it is economical, safe and locally available [11]. The powdered formulation based on essential oil and clay has advantage of being safe, easy to apply and conserve. On the other hand, it can be prepared on site with local materials and farmers could be easily trained to the manufacture of such fungicide. Evaluation of the effect of powdered formulation on sorghum seed germination, seedling growth and determination of its shelf life in different containers will be the topics in future research studies.

**Conclusion**
At the end of this study we can notice that the clay powder from koro have good properties of adsorption because it was rich in smectite and montmorillonite. The aromatization of this clay powder by essential oil of *C. citratus* and *L. mulitiflora* had shown a good efficacy in seed treatment against seed borne fungi. Our results indicated that in seed treatment the efficacy of aromatize clay powder was compared to chemical product used in the control of fungi in seed treatment. Aromatize clay powder with essential oil can be an alternate solution for chemical product on seed treatment.

**Acknowledgements**
The authors would like to express their cordial thanks to the Intra-ACP mobility project HAAGRIM and Plants Clinic of University of Nazi BONI for financial support and laboratory facilities to conduct this research.

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