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Antifungal activity of *Hyptis spicigera* (Lamiaceae) extracts and essential oils of *Cymbopogon citratus* (Poaceae) and *Cymbopogon giganteus* against the growth of *Aspergillus* strains isolated in Burkina Faso

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Abstract: *Antifungal activity of local plant extracts on the growth of seven reference and local isolated strains of Aspergillus was tested. The extracts were composed of essential oils (E.O.) from Cymbopogon citratus, Cymbopogon giganteus and Hyptis spicigera as well as extracts from roots, leafy branches and inflorescences of Hyptis spicigera with organic solvents. The antifungal test was done by disc diffusion method with the application of different concentrations of each type of extract. The results showed that inhibitory action of essential oils was concentration-dependent effect and also strain-dependent. The inhibition diameters of the local strains BfaS0 and BfaS1 were respectively 46,5 mm and 20,5 mm for the E.O. of Cymbopogon citratus at 8 mg of the E.O. concentration. For E.O. of Cymbopogon giganteus, the inhibition diameters were 25,5 mm and 19,0 mm respectively for local strains BfaS0 and BfaS1.*

Keywords: *Antifungal Activity, Cymbopogon spp,Hyptis spicigera, Aspergillus flavus, Aflatoxin*

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I. INTRODUCTION

Aspergillus are microscopic fungi that contaminate crops in fields or during storage in silos or granaries [1]. When climatic conditions are favorable, some strains of *Aspergillus* genus produce aflatoxins which are secondary metabolites known to be highly carcinogenic, immunosuppressive and teratogenic [2].*Aspergillus flavus*, *A. parasiticus* and *A. nomius* are the best known and have been the subject of several research studies that have demonstrated their ability to produce aflatoxins [3], [4], [5][6]. Mycotoxins are detected in a wide range of food products such as Oilseeds, cereals, meat, spices and milk from mammals fed on contaminated foods [7], [8], [9]. Aflatoxins are the most carcinogenic natural substances in biotoxins and are classified in Group I by the International Agency for Research on Cancer [10]. In Burkina Faso, an extensive research program is being conducted to reduce post-harvest aflatoxin production. For example, at Ouagadougou University, work by Nikiéma (1995) [11] and Sanou (2000) [8], showed high levels of aflatoxins in maize (Zea mays), oilseeds including groundnuts (*Arachishypogea*) and their derivatives in the western region of the country. In 2011 and 2012, our work allowed us to isolate and characterize some local strains of *Aspergillus spp* and their ability to produce aflatoxins [12], [13]. Considering these interesting results and also the mistrust regarding the overuse of chemical products [14], [15], more and more researches are interested to plants and their different types of extracts. In the same way we conducted the present study on the evaluation of the antifungal activity of local plant extracts on the growth of strains of *Aspergillus spp*. previously isolated and characterized.

II. MATERIAL AND METHODS

1. Vegetable material as *Hyptis spicigera* (Lamiaceae) collection and conservation

The plant raw material has been harvested in Ouagadougou in the area around dam N °1 for *Hyptis spicigera*. Roots, leafy branches and inflorescences of the plant have been harvested. Plant raw material have been dried at ambient temperature, protected from the sun and then finely powdered and packaged in plastic bags and protected from light and moisture.

2. Essential oils of Cymbopogon citratus and Cymbopogon giganteus

The essential oils (E.O.) were extracted by hydro-distillation using a Clevenger type apparatus. At the end of the extraction the supernatant oil above the water which is frozen in order to allow the recovery of the oil which remains liquid.

3. **Microorganisms**

Reference strains of *Aspergillus spp*. were graciously offered by the USDA-Research, Education and Economics Agricultural Research Service and the CDC Atlanta (USA). They have the following characteristics: Strains NRRL 5862 (*A. parasiticus*, highly aflatoxigenic) and NRRL 484 (*A. flavus*, non- aflatoxigenic) was from USDA whereas strains B4571 (*A. parasiticus*) and B5333 (*A. flavus*) were from CDC and their ability to produce aflatoxins had not been studied yet by CDC by the time they were offered.

4. Local *Aspergillus spp.* Isolates

Local *Aspergillus* species isolation and identification have been described in a scientific publication published in the journal "International Journal of Biological and Chemical Sciences 5 (3): 1232-1249, June 2011. Local *Aspergillus* species were isolated from groundnut seeds. The seeds were wet in glassware and left at the ambient temperature (27 to 34°C) until proliferation of mould, from the consortium of moulds grown, *Aspergillus* strains were isolated and purified on bean broth agar by multiple exhausted seeding. Czapek Yeast extract Agar (CYA) slant was used for further purification and identification using the systematic classification of the *Aspergillus* strains based on morphological characters described by Christensen (1981) [16]; Hocking (1982) [17] and Cotty (1993) [18]. Isolates were thereafter grown on *A. flavus* and *A. parasiticus* medium to ascertain if they belong to *A. flavus* or *A. parasiticus* species. Finally local isolates of *Aspergillus spp.* were assessed for their aflatoxigenic potential.

5. Preparation of conidies

Strains were grown on CYA slant for 10 days at 30 ± 1°C. Spores were harvested in sterilised water containing 0.01% (v/v) tween 80 and centrifuged at 4500 g for 20 min. They were re-diluted in sterilised distilled water and centrifuged again at 4500 g for 20 min.

The operation was repeated three times. The number of conidia was estimated by count under microscope using a Nageotte cell. This suspension is used as an inoculum for the antifungal test.

6. Assessment of antifungal activity of biomedical antifungics and extracts from plants tested

The antifungal activity of plant extracts is tested by the disc diffusion method ([19], [20] on natural coconut agar (CA) medium. Different concentrations per disc of each type of extract are used: 50µg, 100µg, 200µg, 400µg, 800µg, 1000µg, 2000µg, 4000µg, 8000µg. One hundred (100) µl of the conidia inoculum is applied onto the entire surface of the Petri dishes containing the coconut agar medium. The discs (2 to 3) are then deposited onto the Petri dish agar. The whole preparation (Petri dish + CA medium with the discs impregnated with plant extract) is incubated for 48 h at 30 ± 1°C. At the end of incubation, the inhibition zone is determined in mm. Standard biomedical antifungals commonly used for antifungal susceptibility are used as positive control [21]. Dimethyl sulfoxide (DMSO) is used as the negative control.

III. RESULTS AND DISCUSSION

The standards antifungals as well as the essential oils of *Cymbopogon citratus* and *Cymbopogon giganteus* and the extracts of *Hyptis spicigera* were used under the same experimental conditions. The activities of standard antifungals as well as those of *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils obtained by the discs diffusion method are presented in tables 1, 2 and 3.

Among all biomedical standard antifungals, Nystatin has the strongest inhibition potential for all strains studied, followed by Amphotericin B. Thus, the inhibition diameter of Nystatin is 29 mm, twice the Amphotericin B one (14.5 mm) for the local strain type *Aspergillus niger* (BfaS0) (table 1, graph 1, figure 2). This trend is identical for all other strains studied. Graph 1 shows a regular decreasing sensitivity to biomedical standard antifungals depending on the strain in the following order: BfaS0 - NRRL 5862 - CDC 484 - BfaS1 - BfaS5 - CDC 5333 to NRRL 4571. Strain NRRL 4571 exhibits the highest resistance. Miconazole have the lower effective antifungal susceptibility on the seven (07) strains studied (table 1, figure 1, figure 3). Similar study on *Aspergillus fumigatus* and *A. niger* in India in patients with pulmonary tuberculosis [20] showed an antifungal activity of *citratus* and *C. martinii* essential oil on both species. Indian study showed that Miconazole nitrate had the same antifungal susceptibility as *C. citratus* essential oil on the two strains studied.

However, our study indicates that Miconazole is the least effective antifungal standard of the five (05) ones used against the seven (07) strains studied (table 1, graph 1). This could be explained by the concentration of miconazole used but also by the resistance capacity of the different species of *Aspergillus* studied. In addition *Aspergillus species* of our study are isolated from peanut seeds and not in human with pulmonary tuberculosis.

1. Inhibitory action of C*ymbopogon citratus* essential oil (Poaceae)

From 50 to 100 µg the essential oil of *Cymbopogon citratus* showed no inhibitory action on the seven strains studied. From 200 µg, growth began to decrease for the strain BfaS0, *Aspergillus niger* type. At 400 µg,

in addition to the BfaS0 strain, the growth of *Aspergillus parasiticus* NRRL5862 slowed down. At 800 µg, inhibition was observed with diameter of 13 mm and 19,5 mm respectively for *Aspergillus niger* BfaS0 and *A. flavus* CDC 5333 (table 2) At 2000 µg, an inhibition was observed for all the strains except the strain NRRL 4571 for which inhibition began from 4000 µg of *Cymbopogon citratus* essential oil. For strain BfaS1 the inhibition diameter is 16,5 mm (figure 4). At 4000 µg and 8000 µg, an inhibition was observed for all the seven (07) strains with a good increasing inhibition diameters range from 15,5 mm to 33 mm. Following all these observations, it can be inferred that the susceptibility depend on strain and on essential oil concentration.

2. Inhibitory action of C*ymbopogon giganteus essential oil* (Poaceae)

From 50 to 2000 µg, the essential oil of *Cymbopogon giganteus* showed no inhibitory action on the seven strains studied. At 4000 µg inhibition was observed with 13 mm to 19,5 mm diameter for all the strains except the local strain of *Aspergillus flavus* BfaS1 At 8000 µg and 10000 µg an inhibition was observed for all seven (07) strains with a good increasing inhibition diameters range from 19 mm to 31 mm (graph 5) All these observations allowed to tell that the susceptibility of strains depend on essential oil concentration and on strain nature itself.

3. Comparison of inhibitory action of standard biomedical antifungals and essential oils of *C. citratus* and *C. giganteus*

Graphics 1, 2, 3, and figure 5 illustrate comparisons Graphic 1 shows the comparison among standard biomedical antifungals. A regular decreasing sensitivity of strains for standard biomedical antifungals is registered. Nystatin has the strongest inhibition potential while Miconazole has the lowest one.

Graphic 2 compare *Cymbopogon citratus* and *C. giganteus* essential oils inhibitory action at the same concentration (4 mg/disc). *C. citratus* E.O. at this concentration inhibited around more than two times comparing to *C. giganteus*, the growth of following strains: BfaS5, BfaS1, NRRL 5862 and CDC 484. The conclusion is that at an equal concentration (4 mg/disc) the inhibitory action of *C. citratus* essential oil is greater than *C. giganteus* E.O. There is also a relative resistance of the local strain BfaS1 to the E.O. of *C.giganteus* because no inhibitory action has been exhibited at 4mg/disc.

Graphic 3 compare the inhibitory action of standard biomedical antifungals references and those of essential oils of *C. citratus* and *C. giganteus*. This graphic confirm that the inhibitory action of the essential oil of *Cymbopogon citratus* at 4 mg per disc is greater than *C. giganteus* at the same concentration. Figure 5 also confirmed this statement with the big difference of inhibition diameter of the two E.O. at 8 mg/disc (46,5 mm for *C.citratus* versus 25,5 mm for *C. giganteus*). This graphic 3 also shows that *C. citratus* inhibitory action is also greater than all standards biomedical antifungals for five of seven strains except the antifungal Nystatin, whose inhibitory action is higher for two of the local strains: BfaS0 and BfaS1. As we are particularly interested to the resistance of two local *Aspergillus flavus* strains isolated, it is clear that BfaS1, despite of its good susceptibility to standards biomedical antifungal and essential oils, is relatively more resistant than BfaS5. This relative resistance can be linked to its aflatoxin production capacity.

4. Inhibitory action of essential oil and hydro-alcoholic, dicloromethanolic, methanolic, hexanic extracts of *hyptis spicigera* (Lamiaceae)

From 50 to 20 000 µg, the essential oil of *Hyptis spicigera* showed no inhibitory action on the seven strains during the present study. Hydro-alcoholic, dichloromethanolic and methanolic extracts of the leafy stem and the flowers tested at 1000µg, 2000µg, 4000µg, 8000µg, and 10000µg showed no inhibitory activity on the seven strains studied. The organic extracts (hexan) of the leafy stem and the flowers tested at the concentrations of 1000 µg, 2000 µg, 4000 µg showed no inhibitory action on the seven strains studied. In contrary, the same extracts tested at higher concentrations of 8000 µg and 10 000 µg caused the growth slowing of BfaS0 strain through a decreasing of the density of the mycelium. For the six other strains tested, no inhibition was observed. According to the literature, the majority of investigations concern the insecticidal properties of *Hyptis spicigera*. [22]; [23] In the present study, the inhibitory action against the growth of the BfaS0 strain observed for 8 and 10 mg is an advance and must be studied deeper, especially as it is a plant easily found even at the edge of dams in the city.

IV. CONCLUSION

This study showed a dependent-concentration effect of essential oils of *Cymbopogon citratus* and *C. giganteus* against*Aspergillus* strains studied whether they are aflatoxin-producing or not. Unlike the essential oil of the two species of *Cymbopogon*, the E.O. of *Hyptis spicigera* and its various hydro-alcoholic, dichloromethanolic, methanolic and hexanic extracts did not show a significant inhibitory action on the seven strains studied. However, it can be noted that the local aflatoxin-producing *Aspergillus flavus* BfaS1 strain showed less susceptibility depending on the concentration of essential oil *C. giganteus* until 4 mg per disc. In perspective this study results are promising and require the work continuation by:

- investigating the sensitivity of the two local strains of *Aspergillus* (BfaS5 and BfaS1) to the essential oils of *Cymbopogon. citratus* and *C. giganteus*
- expanding the study to strains responsible for aspergillosis in humans.
- conducting a toxicological study of these essential oils in order to use them for pre and post-harvest preservation and also considering their use in the treatment of human aspergillosis.

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TABLES

		Diameter (mm) of standard biomedical antifungals inhibition zone per strain						
Biomedical antifungals	**Symbol**	**BfaS0**	**BfaS1**	**BfaS5**	**CDC 5333**	**CDC 484**	**NRRL 5862**	**NRRL 4571**
Nystatine (100 UI)	NY 100	29,0±0,82	22,3±1,71	21,0±1,15	21,0±1,15	23,3±0,96	23,5±0,58	15,8±1,26
Amphotericine B (20µg)	AMB 20	14,5±0,71	9,0±0,0	13,5±2,12	8,5±0,71	14,0±0,0	15,5±0,71	10,0±0,0
Clotrimosazole (50µg)	CLO 50	9,5±0,71	9,0±0,71	12,5±0,71	9,5±0,71	7,5±0,71	12,5±0,71	9,5±0,71
Fluconazole (100 UI)	FLU 100	0	0	8,5±0,71	0	13,0±1,41	0	0
Miconazole (10 UI)	MCL 10	0	0	0	0	0	0	0

NY 100: Nystatin (100 UI) AMB 20: Amphotericin B (20µg), CLO 50 : Clotrimosazole (50µg),
FLU 100: Fluconazole (100 UI) MCL 10: Miconazole (10 UI)

Table 1: Inhibition diameter of standard biomedical antifungals

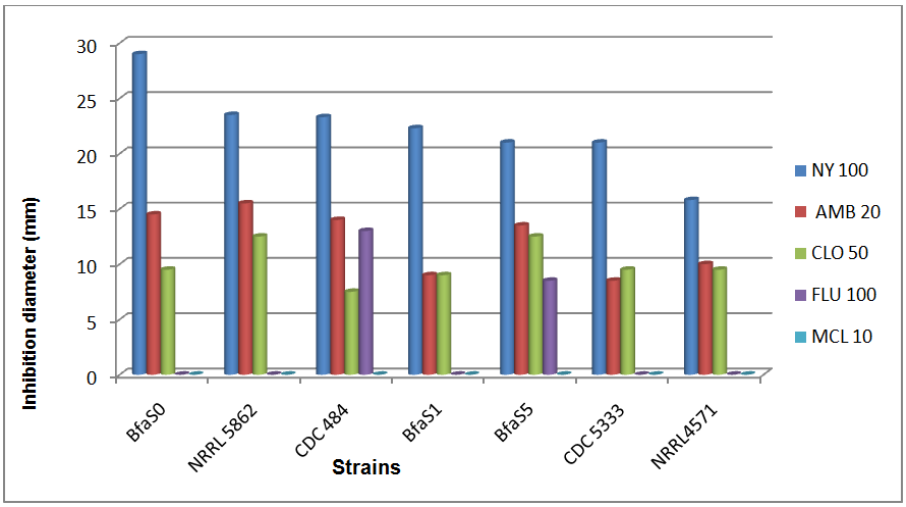
	Diameter (mm) of *Cymbopogon citratus* essential oil inhibition zone per strain						
Strains	**BfaS0**	**BfaS1**	**BfaS5**	**CDC 5333**	**CDC 484**	**NRRL 5862**	**NRRL 4571**
E.O. concentration							
C. citratus 50 et 100 µg	0	0	0	0	0	0	0
C. citratus 200 µg	Growth slowed down	0	0	0	0	0	0
C. citratus 400 µg	Growth slowed down	0,0	0	0,0	0	Growth slowed down	0
C. citratus 800 µg	13 ± 0,0	0,0	0,0	19,5 ± 0,71	0,0	Growth slowed down	0,0
C. citratus 1000 µg	13 ± 0,0	0,0	0,0	21,5 ± 0,71	0,0	Growth slowed down	0,0
C. citratus 2000 µg	17 ± 1,41	16,5 ± 2,12	19,5 ± 0,71	25,5 ± 0,71	10,5 ± 0,71	14,5 ± 0,71	0,0
C. citratus 4000 µg	22,5 ± 0,71	19,5 ± 0,71	26 ± 0,0	29 ± 1,41	24,5 ± 0,71	24,5 ± 0,71	15,5 ± 0,71
C. citratus 8000 µg	46,5 ± 0,71	25,5 ± 0,71	31,5 ± 2,12	41 ± 1,41	31 ± 1,41	33 ± 1,41	20,5 ± 0,71

Table 2: Inhibition diameter of *Cymbopogon citratus* essential oil

	Diameter (mm) of *Cymbopogon giganteus* essential oil inhibition zone per strain						
Aspergillus strains	**BfaS0**	**BfaS1**	**BfaS5**	**CDC 5333**	**CDC 484**	**NRRL 5862**	**NRRL 4571**
E.O. concentration							
C. giganteus 50 µg à 2000 µg	0	0	0	0	0	0	0
C. giganteus 4000 µg	14,5 ± 0,71	0,0	13 ± 0,0	19,5 ± 0,71	13 ± 0,0	13 ± 0,0	13 ± 0,0
*C. giganteus*8000 µg	25,5 ± 0,71	14,5 ± 0,71	19,0 ± 1,41	23,5 ± 0,71	19,0 ± 1,41	19,5 ± 0,71	19,0 ± 1,41
C. giganteus 10000 µg	31 ± 1,41	19 ± 1,41	21 ± 1,41	31 ± 1,41	21 ± 1,41	23,5 ± 0,71	19 ± 1,41

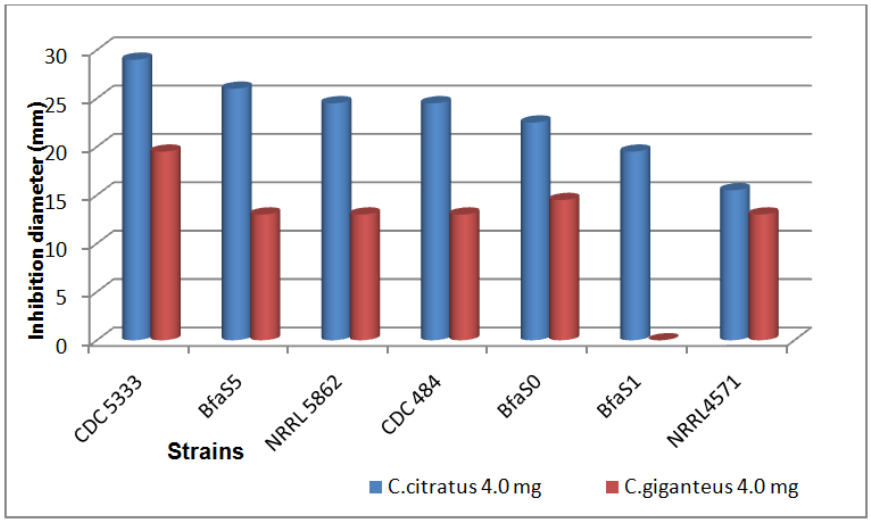
Table 3: Inhibition diameter of *Cymbopogon giganteus* essential oil

GRAPHICS

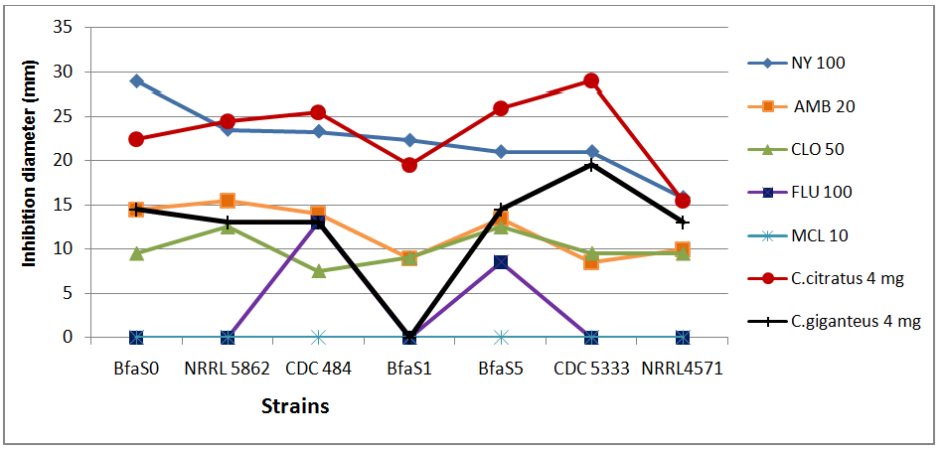


NY 100: Nystatin (100 UI) AMB 20: Amphotericin B (20µg), CLO 50: Clotrimosazole (50µg),
FLU 100: Fluconazole (100 UI), MCL 10: Miconazole (10 UI)

Graph 1: Comparison of standard biomedical antifungals inhibitory action



Graph 2: Comparison of essential oils inhibitory action of *C. citratus* and *C. giganteus*



NY 100: Nystatin (100 UI) AMB 20: Amphotericin B (20µg), CLO 50 : Clotrimosazole (50µg), FLU 100: Fluconazole (100 UI) MCL 10: Miconazole (10 UI)

Graph 3: Comparison of standard biomedical antifungals inhibitory action and essential oils of *Cymbopogon citratus* and *C. giganteus* inhibitory action

FIGURES

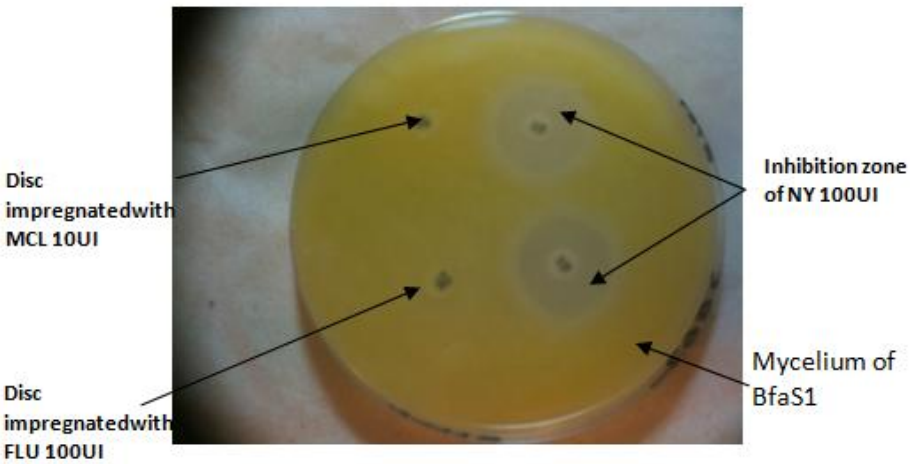


Fig. 1: Antifongigram of Nystatin (100UI), Myconazole (10UI), Fluconazole (100UI) inhibitory action on strain BfaS1

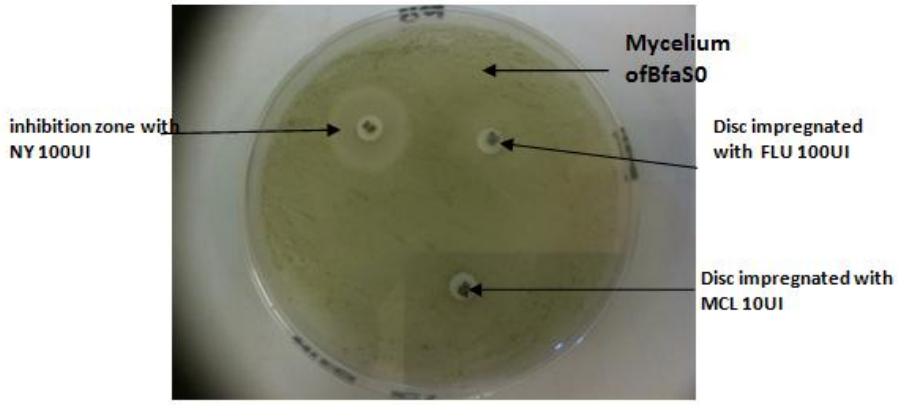


Fig. 2: Antifongigram of Nystatin (100UI), Myconazole (10UI), Fluconazole (100UI) inhibitory action on strain BfaS0

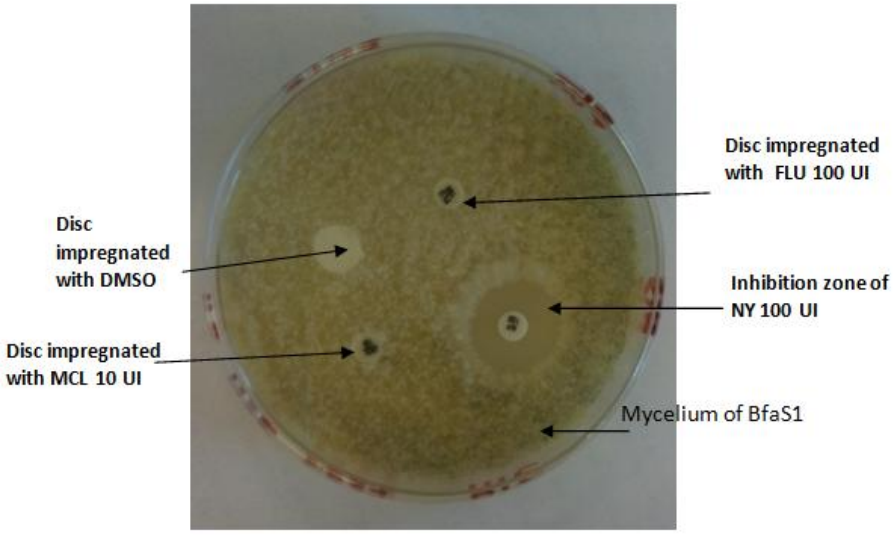


Fig.3: Antifongigram of Nystatin (100UI), Myconazole (10UI), Fluconazole (100UI) and DMSO inhibitory action on strain BfaS1

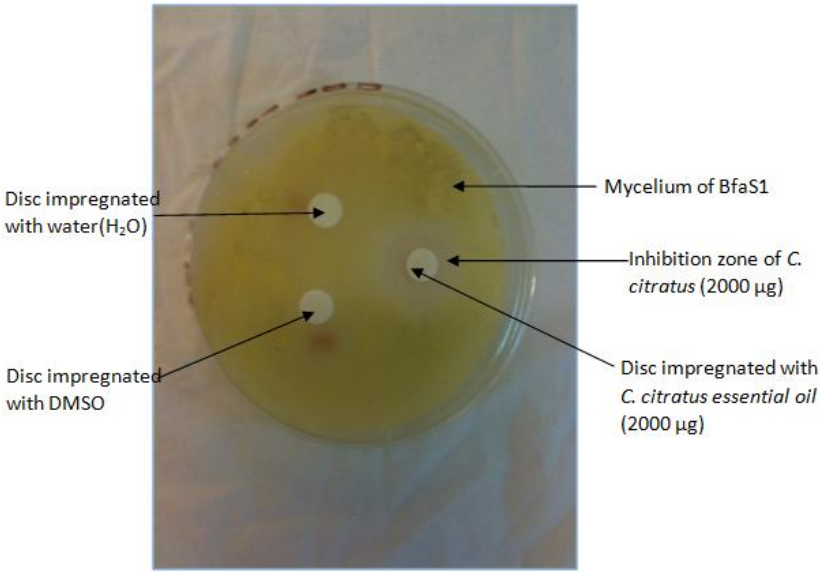


Fig. 4: Antifongigram of *C. citratus* essential oil (2000 µg), H_2O, DMSO inhibitory action on strain BfaS1

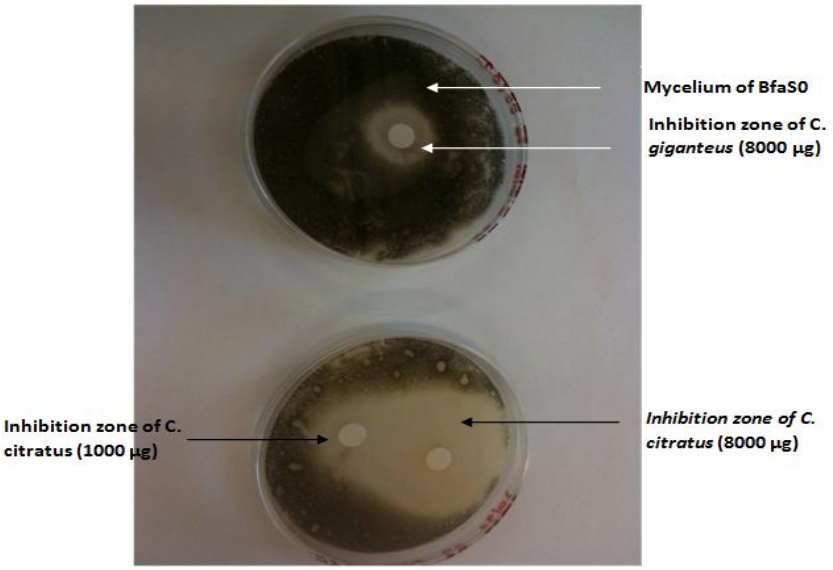


Fig. 5: Antifongigram of essential oils of *C. Giganteus* (8000µg) and *C. citratus* (1000 and 8000 µg) inhibitory action on strain BfaS0

Pane B. Ouattara–Sourabie. "Antifungal activity of Hyptis spicigera (Lamiaceae) extracts and essential oils of Cymbopogon citratus (Poaceae) and Cymbopogon giganteus against the growth of Aspergillus strains isolated in Burkina Faso." IOSR Journal of Pharmacy (IOSR-PHR) 7.7 (2017): 17-24.