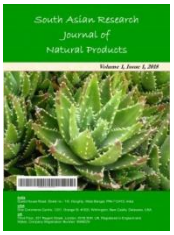

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# *In vitro* Fungitoxicity of Four Essential Oils on Sugarcane Smut *Sporisorium scitamineum* Piep., in Côte d'Ivoire

**Kouamé Konan Didier[1*], N'guessan Aya Carine[2], Kassi Koffi Fernand Jean-Martial[1], Kouamé Koffi Gaston[2], Yao Kouadio Jacques-Edouard[1], Yobouet Aya Ange Naté[1], Koné Daouda[1] and Zouzou Michel[1]**

*[1] Laboratory of Plant Physiology and Plant Pathology, UFR (Faculty) of Biosciences, University Félix Houphouët-Boigny, 22 BP 582, Abidjan, Côte d'Ivoire.*
*[2]UFR (Faculty) of Biological Sciences, University Péléforo Gon Coulibaly, Côte d'Ivoire.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author KKD designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors NAC and KKFJM managed the analyses of the study and performed the statistical analysis. Authors KKG, YKJE and YAAN collected the data and managed literature searches. Authors KD and ZM supervised the study and learn the final version of the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** Sugarcane, *Saccharum officinarum* (Poaceae) is an important industrial crop in Côte d'Ivoire. However, many biotic constraints such as smut du to *Sporisorium scitamineum* lead to yield limitation. The management of this pathogen includes thermotherapy, which is not always effective and the use of systemic fungicides which have a negative impact on human health and biodiversity. This study was to evaluate the fungitoxicity of four essential oils on *Sporisorium scitamineum*, in Côte d'Ivoire.

**Corresponding author: Email: didykonan@yahoo.fr;*

**Study Design:** Therefore, an *in vitro* control trial of *Sporisorium scitamineum* isolates with essential oils of *Ocimum gratissimum*, *Zingiber officinale*, *Cymbopogon citratus* and *Melaleuca quinquenervia* was performed. The positive control used was propiconazole, a synthetic fungicide (triazole family). Different concentrations of the essential oils and the systemic fungicide were tested on mycelial growth and sporulation of two fungal strains S1 and S2 of *Sporisorium scitamineum* isolated from sugarcane varieties CP921167 and VMC93282.
**Place and Duration of Study:** The study was performed in 2014-2015 harvest season at the sugar bowl of Ferké in Northern Côte d'Ivoire.
**Methodology:** The effect of essential oils was compared to that of propiconazole. Thus, for the oils, 100 ml of different culture media at doses of 250, 500, 1000, 2000 and 4000 µl/l were prepared. Also, the media of propiconazole were used at four five doses (20, 25, 50, 100, 150 µl/l) prepared from 100 ml of REFERENCE, the positive control solution. The Petri dishes were incubated for 30 days, and the mycelial growth was assessed every 48hrs according to perpendicular axes at the base of each Petri dish. The radial growth measurements were used to calculate the inhibition rates of each product at the assessed doses, and the IC50 and IC90 of different assessed products were determined with ED50 plus V1.0 software.
**Results:** The results showed that the effect of essential oils of *Ocimum gratissimum* and *Cymbopogon citratus* as well as propiconazole inhibited the mycelial growth and sporulation of both smut strains at all concentrations. The essential oil of *Zingiber officinale* proved to be moderately inhibitory compared to that of *Melaleuca quinquenervia*.
**Conclusion:** These essential oils could be applied *in vivo* to assess their effect on the incidence of *Sporisorium scitamineum* on sugarcane cultivation with a view of a sustainable management system of sugarcane diseases, for environmental protection and human health.

*Keywords: Saccharum officinarum; Sporisorium scitamineum; smut; essential oils; sustainable management.*

## 1. INTRODUCTION

Sugarcane, *Saccharum officinarum* L. (Poaceae) is an industrial crop introduced in Africa in the XVII th century. It represents the largest harvest volume in the world [1]. This monocotyledon is very important both in terms of agri-food and energy. Between 2010 and 2011, the world sugarcane yield increased by 3% with a volume of 165300000 t [2]. More than three quarters of the world sugar production stems from sugarcane, the rest is produced from sugar beet [3].

In Côte d'Ivoire, sugarcane plays an important role in the economy. In terms of surface area, it occupies 25400 ha over a land area of 61400 ha exploited by sugar industries [4]. Côte d'Ivoire ranks 53rd globally and 16th in Africa in terms of sugar production [5]. Within the WAEMU area, sugar production amounts to 214000 t of finished sugar. This plant is mainly grown in the North and Central West of the country and is however subject to many biotic constraints which lead to its yield limitation. Among these threats, smut caused by *Sporisorium scitamineum* is responsible for significant damage in Africa [6,7]. This microscopic fungus causes growth disturbances in the host plant, resulting in significant drops in yield. Losses caused by smut thus vary from 20 to 30% of the final yield of sugarcane [8]. Several varieties of sugarcane, the most sensitive of which record more significant losses [9].

The control methods for reducing the impact of the disease amount to plant sanitation by the manual uprooting of whips, preventive treatment of cuttings, use of resistant varieties and application of systemic fungicides [9,10]. Most of the currently used synthetic fungicides directly affect vital life functions such as respiration, sterol biosynthesis or cell division. This mode of action can lead to health risks for humans and non-target organisms. The search for new, non-harmful alternative molecules is becoming paramount for producers concerned about the health of the consumer and eager to be competitive in all markets. Essential oils extracted from aromatic plants are an important asset in this new quest. The work carried out on the antifungal activity [11,12] and on the insecticidal activity of the essential oil extracted from *Ocimum gratissimum* leaves revealed its ability in the control of plant pests [13]. The general objective of this study is to evaluate the fungitoxic potential of essential oils of *Ocimum*

*gratissimum* L., *Melaleuca quinquenervia*, L., *Cymbopogon citratus* L. and *Zingiber officinale* Rosc. on *Sporisorium scitamineum* (formerly *Ustilago scitaminea* Syd.), causal agent of sugarcane smut in Côte d'Ivoire.

## 2. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Fungal material

Two strains of the fungus *Sporisorium scitamineum* (S1 and S2) were isolated respectively from the culms of two sugarcane varieties (CP 921167 and VMC 93282) showing symptoms of smut.

#### 2.1.2 Essential oils

Essential oils extracted from the leaves of *Ocimum gratissimum* L. *Melaleuca quinquenervia* L., *Cymbopogon citratus* L. and rhizomes of *Zingiber officinale* Rosc. were used. These essential oils were obtained by steam distillation in a Clevenger-type apparatus.

#### 2.1.3 Synthetic fungicide

Propiconazole, the positive control, is a systemic fungicide of the triazole family used at 250 g/L, was assessed for *Sporisorium scitamineum* growth control and sporulation.

### 2.2 Methods

#### 2.2.1 Isolation of the fungus and production of monosporic strains

Sugarcane stems with characteristic symptoms of smut were removed and cut into fragments. These fragments were disinfected with alcohol at 70° and then soaked in 5% sodium hypochlorite solution for 3 minutes before a series of thrice washes for 3 minutes in sterile distilled water. The stem fragments thus disinfected were seeded on an agar-based (2%) and sterile distilled water culture medium. The seeded Petri dishes were incubated for 72 hours. The purification and multiplication of the fungal strains obtained were carried out on a PDA (Potato Dextrose Agar) culture medium.

After obtaining pure strains having sporulated, a fungus culture from individualised spore was carried out on the PDA culture medium. To this end, a spore solution was prepared with sterile distilled water. Successive ten-fold dilutions were made until a $10^5$ spores/ml solution was obtained. Then, 1 ml of that solution was removed and spread in a Petri dish containing an agar culture medium. After 24 hours of incubation, using an optical microscope, the spores that have germinated were transferred to a PDA culture medium in order to obtain monosporic strains. Images of germinating spores were taken from the Petri dishes using an optical microscope equipped with a camera (AmScope). From these images, and using the ToupView 3.7 software, microscopic observations of the mycelium and teliospores were made.

#### 2.2.2 Production of essential oils

The essential oils of the aromatic plants used during this study were obtained from fresh organs, by saturated steam distillation carried out with the Clevenger-type apparatus for 2 h [14]. This method consists in a conventional distillation in which the plant is not in direct contact with water. Indeed, in the saturated steam distillation, the plant material was placed on a grid and traversed by a stream of water vapor. During the passage of the vapor, through the material, the cells burst and released the essential oil which was driven towards the condenser and the essencer. The separation was done by decantation.

Essential oil's yield is the ratio between the mass of oil extracted and the mass of the plant treated [14]. It is expressed as a percentage and is calculated according to the following formula (1):

$$R = \frac{PB}{PA} \quad (1)$$

Where, R is Oil yield in %; PB is mass of oil in g and PA is mass of the plant in g.

#### 2.2.3 *In vitro* effect of essential oils and chemical fungicide on the radial growth of *Sporisorium scitamineum* strains

The effectiveness of essential oils was compared to that of propiconazole. Thus, for the five essential oils, 100 ml of different culture media at doses of 250, 500, 1000, 2000 and 4000 µl/l were prepared. Also, 5 culture media of 100 ml containing propiconazole at doses of 20, 25, 50, 100, 150 µl/l were prepared from 100 ml of positive control stock solution. After homogenisation, the different culture media were distributed under host in 9-cm diameter sterile

Petri dishes at the rate of 5 dishes per concentration and per product. The control culture medium containing neither essential oil nor synthetic fungicide was also distributed in 5 Petri dishes. A 7-mm diameter mycelial disc of the monospore strain was used to seed the different culture media, either amended or not. The Petri dishes were then incubated for 30 days, where mycelial growth was assessed every 48hrs according to two perpendicular axis drawn at the base of each Petri dish and intersecting in the middle of the explant.

Radial growth measurements were used to calculate the inhibition rates of each product at the assessed doses.

$$Inhibition\ rate = \frac{(C0 - Cn)*100}{C0} \quad (2)$$

Where, *C0 is the average diameter of colonies on the control culture media* and *Cn is average diameter of colonies on culture media amended with essential oils and synthetic fungicide at different concentrations.*

IC50 and IC90 (inhibitory concentrations of 50 p.c. and 90 p.c. of mycelial growth) of the different assessed products were determined with ED50 plus V1.0 software. The inhibition percentages were transformed by the probit method and, from a modeling that integrated the trend curve, the equation of the regression line associated with the log10 function, the very low doses of inhibition were determined.

#### 2.2.4 *In vitro* effect of essential oils and propiconazole on sporulation of *Sporisorium scitamineum* strains

The spores contained in the fungal filtrate were observed under an optical microscope and the average number was determined using a Malassez hematimeter after two successive counts.

The inhibition rate of sporulation (IRs) of each product at the assessed doses was determined according to the following formula:

$$IRs = \frac{(N0 - Nc)*100}{N0} \quad (3)$$

Where, N0 is the number of spores estimated in the control Petri dishes and Nc the number of spores estimated in Petri dishes amended with essential oils or propiconazole.

### 2.3 Data Analysis

The analysis of variance (ANOVA) with two classification criteria was used to assess the combined effect of the product and applied dose on mycelial growth reduction rate and sporulation of both *Sporisorium scitamineum* strains. The Newman-Keuls test made it possible to compare the average values of the parameters assessed at 5% α risk in order to classify the different products and the incorporated doses according to their toxicity on *Sporisorium scitamineum* growth. Statistical analyses of the data were carried out with XLSTAT version 7.1 software.

## 3. RESULTS

### 3.1 Microscopic Characteristics of the Isolated Fungus

The microscopic observation of the fungus isolated and grown on the PDA culture medium revealed a septate mycelium shown in Fig. 1 and coarsely spherical or teliospore unicellular formations shown in Fig. 2. This microscopic description of the fungus is similar to that of the organs of *Sporisorium scitamineum*, pathogen responsible for sugarcane smut.

### 3.2 Yield of Essential Oils Extracted

Essential oils yield was function of plants and organs used. Highest yields were obtained from plants leaves and rhizomes (for *Zingiber officinale*). Thus, *Ocimum gratissimum* had the highest yield (1.2%). *Melaleuca quinquenervia* and *Cymbopogon citratus* had yields of 0.9 and 1.05% respectively. *Zingiber officinale's* essential oil extraction yield was the lowest with 0.7%.

### 3.3 Antifungal Activity of Essential Oils and Synthetic Fungicide on *Sporisorium scitamineum* Mycelial Growth

The inhibition percentages of *Sporisorium scitamineum* mycelial growth are shown in Table 1. The analysis of the results showed that the essential oils of *Ocimum gratissimum* and *Cymbopogon citratus* were the most effective in reducing the mycelial growth of both fungus strains. A complete inhibition at all tested concentrations (250 to 4000 ppm) was observed.

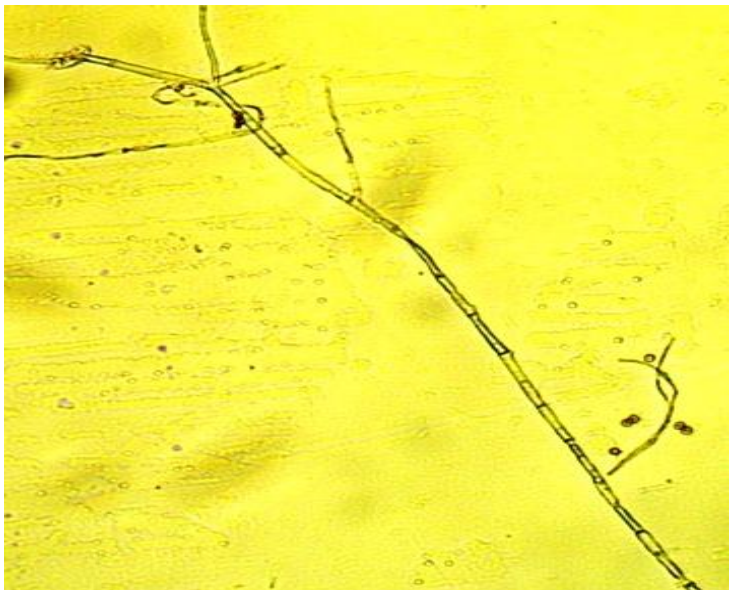

**Fig. 1. Septate mycelial filament of *Sporisorium scitamineum* (M X 400)**

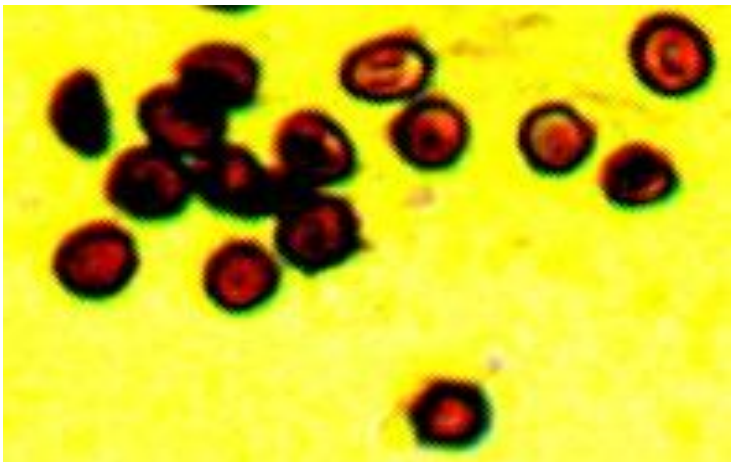

**Fig. 2. Teliospores mass of *Sporisorium scitamineum* (M X 400)**

The effect of these two essential oils was statistically identical to that of the synthetic fungicide, propiconazole. *Melaleuca quinquenervia* oil showed low antifungal activity on the 2 tested strains compared to all the tested oils. Total inhibition was observed only at a concentration of 4000 ppm for both strains.

As for *Zingiber officinale* oil, total inhibition of growth was observed at concentrations of 2000 ppm and 1000 ppm for strains S1 and S2 respectively.

### 3.4 Determination of IC50 and IC90 of the Assessed Products

In the presence of the essential oil of *Ocimum gratissimum* and *Cymbopogon citratus*, a total inhibition of the mycelial growth of both fungal strains (S1 and S2) was observed at 250 ppm, the lowest assessed concentration. As for the essential oils of *Zingiber officinale* and *Melaleuca quinquenervia* their fungitoxicity was relatively low on the mycelial growth of *Sporisorium scitamineum* strains S1 and S2. However, as from 1000 ppm, *Zingiber officinale* inhibited the fungus growth (Table 2). Propiconazole significantly reduced the growth of these two strains with very low IC50 values. Thus, 0.159 ppm and 46.244 ppm of this fungicide reduced 50% and 90% of the mycelial growth of strain S1 whereas with strain S2, a 90% reduction in mycelial growth was obtained with a lower concentration (29.168 ppm).

### 3.5 Effect of Essential Oils and Propiconazole on Spore Production

The results in Table 3 show that the essential oils of *Ocimum gratissimum* and *Cymbopogon citratus* caused a total inhibition of the sporulation of strains S1 and S2 at all the assessed doses. The sporulation stage was also sensitive to *Zingiber officinale* (65.6% and 97.1% inhibition rate for strains S1 and S2 respectively at 250 ppm) and *Melaleuca quinquenervia* oil (47.8% and 98.7% inhibition rate for strains S1 and S2 respectively at 250 ppm). Propiconazole was also very effective on sporulation of *Sporisorium scitamineum* strains.

**Table 1. Effect of essential oils and propiconazole on the mycelial growth of *Sporisorium scitamineum* strains S1 and S2**

| Inhibition rates of mycelial growth (%) | | | |
|---|---|---|---|
| **Treatments** | **Concentrations (ppm)** | **Strain S1** | **Strain S2** |
| *Ocimum gratissimum* | 250 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| | 500 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| | 1000 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| | 2000 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| | 4000 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| *Zingiber officinale* | 250 | 0.0± 0.0 d | 0.0 ± 0.0 d |
| | 500 | 0.0± 0.0 d | 0.0 ± 0.0 d |
| | 1000 | 60.0 ± 24.5 b | 100.0 ± 0.0 a |
| | 2000 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| | 4000 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| *Cymbopogon citratus* | 250 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| | 500 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| | 1000 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| | 2000 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| | 4000 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| *Melaleuca quinquenervia* | 250 | 0.0 ± 0.0 d | 0.0 ± 0.0 d |
| | 500 | 0.0 ± 0.0 d | 0.0 ± 0.0 d |
| | 1000 | 9.2 ± 9.2 cd | 20.0 ± 20.0 c |
| | 2000 | 26.6 ± 7.8 c | 92.7 ± 7.3 a |
| | 4000 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| Propiconazole | 20 | 82.8 ± 17.2 a | 88.9 ± 11.1 a |
| | 25 | 84.0 ± 16.0 a | 88.9 ± 11.1 a |
| | 50 | 89.9 ± 10.1 a | 90.6 ± 9.4 a |
| | 100 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| | 150 | 100.0 ± 0.0 a | 100.0 ± 0.0 a |

*Means followed by the same letter are statistically identical at α = 5% threshold (Newman-keuls test); Average ± standard deviation*

**Table 2. Doses of essential oils and synthetic fungicide inhibiting 50% and 90% of *Sporisorium scitamineum* mycelial growth**

| **Treatments** | **Strain S1** | | **Strain S2** | |
|---|---|---|---|---|
| | **$IC_{50\ (ppm)}$** | **$IC_{90\ (ppm)}$** | **$IC_{50\ (ppm)}$** | **$IC_{90\ (ppm)}$** |
| *Ocimum gratissimum* | - | - | - | - |
| *Zingiber officinale* | 1477.63 | 2952.1 | 1154.26 | 2737.23 |
| *Cymbopogon citratus* | - | - | - | - |
| *Melaleuca quinquenervia* | 2393.21 | 3869.92 | 1801.63 | 3150.85 |
| Propiconazole | 0.159 | 46.244 | 0.0046 | 29.168 |

*- Not determined*

**Table 3. Effects of oils and synthetic fungicide on sporulation of *Sporisorium scitamineum* strains S1 and S2**

| Treatments | Concentrations (ppm) | Production of strain spores | | | |
|---|---|---|---|---|---|
| | | Strain S1 | | Strain S2 | |
| | | Mean number of spores x ($10^5$) | Inhibition rate of sporulation (%) | Mean number of spores x ($10^5$) | Inhibition rate of sporulation (%) |
| **Control** | | 52.8 | | 166.0 | |
| *Ocimum gratissimum* | 250 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| | 500 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| | 1000 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| | 2000 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| | 4000 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| *Zingiber officinale* | 250 | 20.6± 0,11i | 65.6± 0.08 j | 3.6± 0.05 e | 97.1± 0.28 c |
| | 500 | 7.3± 0.05 g | 67.4± 0.38 i | 8.2± 0.08 h | 85.2± 0.07 e |
| | 1000 | 1.8± 0.14 d | 96.1± 0.11 d | 0± 0.0 a | 100± 0.0 a |
| | 2000 | 0±0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| | 4000 | 0±0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| *Cymbopogon citratus* | 250 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| | 500 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| | 1000 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| | 2000 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| | 4000 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| *Melaleuca quinquenervia* | 250 | 3.4± 0.08 e | 47.8± 0.17 k | 1.1± 0.11b | 98.7± 0.26 h |
| | 500 | 1.7± 0.17 d | 73.3± 0.37 g | 1± 0.11 b | 98.8± 0.1 b |
| | 1000 | 1.5± 0.08cd | 75.5± 0.23 f | 1.6± 0.44 f | 98.3± 0.08 b |
| | 2000 | 1.8± 0.17 d | 71.2± 0.54 h | 1.4± 0.11 c | 98.4± 0.17 b |
| | 4000 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| Propiconazole | 20 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| | 25 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| | 50 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| | 100 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |
| | 150 | 0± 0.0 a | 100± 0.0 a | 0± 0.0 a | 100± 0.0 a |

*Means followed by the same letter are statistically identical at α = 5% threshold (Newman-keuls test); Average ± standard deviation*

## 4. DISCUSSION

Essential oils yield of different plants extracted by steam distillation range from 0.7 to 1.2%. Two distinct groups are defined: Group I containing plants whose essential oil yield is less than 1%. In this group, we have *Zingiber officinale* (0.7%) and *Melaleuca quinquenervia* (0.9%). Group II, gather plants with a yield greater than 1%. It includes *Ocimum gratissimum* (1.2%) and *Cymbopogon citratus* (1.05%).

For essential oil of *Ocimum gratissimum*, our results are similar to those of [15], which obtained 1.24% of essential oils from leaves collected in Benin. A yield of 0.60% has been reported in other study with *Ocimum gratissimum* in Cameroon [16]. Essence extracted from *Zingiber officinale*'s rhizomes are very close to those of [17]. Djibo [18] obtained a similar yield of essential oil about 1.4% with *Cymbopogon citratus*. For *Melaleuca quinquenervia*, Ramanoelina et al. [19] showed that the yield of essential oil is between 0.2 and 1.1%, with an average of 0.8%.

The effect of essential oils and synthetic fungicide on the mycelial growth and sporulation of both strains of *Sporisorium scitamineum* showed different effectiveness depending on the products and doses incorporated in the culture medium. This effect was proportional to the assessed doses.

*Ocimum gratissimum* and *Cymbopogon citratus* oils proved to be effective in inhibiting

*Sporisorium scitamineum* mycelial growth and sporulation. Their activities were similar to that of Propiconazole, the synthetic fungicide. Those of *Zingiber officinale* and *Melaleuca quinquenervia* were less fungitoxic.

Moharam et al. [20] also highlighted the antifungal effect of plant extracts on *Sporisorium ehrenbergii* Vanny the causal agent of long smut on sorghum in Egypt. These Works showed that water extract of rheum (*Rheum rhabarbarum*) and common walnut (*Juglans regia*) at 1% completely inhibited teliospore germination and caused highest inhibition of the mycelium growth of two strains of this fungus.

The antifungal activities observed with these essential oils might be due to the nature of the chemical composition of each oil. Previous work carried out by Kassi [21] on the composition of the essential oil of *Ocimum gratissimum* harvested in Côte d'Ivoire has shown that it is predominantly rich in thymol (46.1%) and ɣ-terpinene (17.6%). Thymol is a phenolic compound with potent antimicrobial activity [22]. The presence of this compound in this essential oil would partly justify its antifungal activities observed during this study. The work of [23] conducted in 2010 for the control of wood-rotting fungi showed the effectiveness of *Thymus ciliatus* (Desf.) Benth mostly composed of thymol (44.2%).

The antifungal effectiveness of low-dose *Cymbopogon citratus* essential oil is confirmed by the work of [24]. In this work, the essential oil of *Cymbopogon citratus* at 400 ppm and 600 ppm completely inhibited the mycelial growth of *Bipolaris oryzae* and that of *Pyricularia oryzae* as from 200 ppm.

Concerning *Zingiber officinale* oil, inhibitory activity was observed at concentrations greater than 500 ppm. This is best explained by the results of [25], which showed that the *Zingiber* extract had an antibacterial activity, and that the inhibition of bacterial growth was dose-dependent.

The essential oil of *Melaleuca quinquenervia* was the one whose antifungal effectiveness was expressed at very high doses (2000 and 4000 ppm) [26], finded an inhibitory dose of 7000 ppm with this essential oil on *Deightoniella torulosa* (Syd.) Ellis. According to Séri Kouassi et al. [27], the antifungal activity obtained at these high doses of essential oil might be due to the low toxicity of its main components which are 1,8-cineole, viridiflorol and α-pinene.

## 5. CONCLUSION

The extraction yield of essential oils is the function of the plants and organs used. *Ocimum gratissimum* had the highest yield followed by *Cymbopogon citratus* and *Melaleuca quinquenervia*. The lowest essential oil extraction yield was obtained with *Zingiber officinale*. Among the plant extracts used, essential oils extracted from the leaves of *Ocimum gratissimum* and *Cymbopogon citratus*, at all concentrations, showed a strong antifungal activity against *Sporisorium scitamineum* Piep., both on its growth and on its proliferation organs (spores). As for the essential oil of *Zingiber officinale*, the dose capable of inducing a fungitoxic effect was obtained at 1000 ppm. That of *Melaleuca quinquenervia* has not proved really effective against both fungal strains studied. It would be interesting to check the synergistic effect of the assessed essential oils and also to test the effectiveness of these extracts in disinfection of artificially infected cuttings so as to assess their ability to control *Sporisorium scitamineum* in the host plant.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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