Agricultural Sciences, 2023, 14, 1068-1086
https://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

In Vitro Evaluation of the Antifungal Activity of Essential Oils of *Thymus vulgaris* and *Cymbopogon citratus* on Some Strains of *Sclerotinia sclerotiorum*, Agent Responsible for White Rot of Beans

Serge Bertrand Mboussi[1*], Alain Heu[2], Abdou Nourou Kone Nsangou[3], Jules Patrice Ngoh Dooh[4], Zachée Ambang[5]

[1]Laboratory of Biotechnology, University Institute of Technology, University of Douala, Douala, Cameroon
[2]Department of Agriculture and Agropastoral, Higher Technical Teachers Training College, Ebolowa, Cameroon
[3]Applyied Botanic Research Unit, Department of Plant Biology, University of Dschang, Dschang, Cameroon
[4]Department of Biological Sciences, Faculty of Sciences, University of Maroua, Maroua, Cameroon
[5]Biotechnology Laboratory, Phytopathology and Microbiology Unit, University of Yaoundé I, Yaoundé, Cameroon
Email: *smboussi@gmail.com

How to cite this paper: Mboussi, S.B., Heu, A., Nsangou, A.N.K., Dooh, J.P.N. and Ambang, Z. (2023) *In Vitro* Evaluation of the Antifungal Activity of Essential Oils of *Thymus vulgaris* and *Cymbopogon citratus* on Some Strains of *Sclerotinia sclerotiorum*, Agent Responsible for White Rot of Beans. *Agricultural Sciences*, **14**, 1068-1086.
https://doi.org/10.4236/as.2023.148071

Received: May 15, 2023
Accepted: August 25, 2023
Published: August 28, 2023

Copyright © 2023 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Abstract

The present work was aimed to the study of the antifungal activity of essential oils of two aromatic plants against three strains of *Sclerotinia sclerotiorum* responsible for white rot of bean. The two essential oils (EO) of the studied plants: *Thymus vulgaris* (HET) and *Cymbopogon citratrus* (HEC), used at different concentrations: C1 (0.75 ml/ml), C2 (1.5 ml/ml) and C3 (3 ml/ml) presented significant inhibitory activities on the three tested fungal strains namely Bia 1, Bia 2, and Njo 2. Two control treatments (T-: containing no antifungal substance and T+: synthetic fungicide) were also used. The antifungal activity here is related to the inhibition of mycelial growth especially with high concentrations of essential oils. Thus, against *S. sclerotiorum*, HET showed the highest activity comparable to that of T+ (100% inhibition) at all concentrations on the Bia 1 and Bia 2 strains and at concentration C3 on the Njo 2 strain compared to that of HEC, which certainly inhibited the mycelial growth of the different strains considerably (with a maximum of 78.15% on the Njo 2 strain) but not completely. According to their sensitivity, the Bia 2 strain showed a higher sensitivity to essential oils than the others while the Njo 2 strain was more aggressive. On the basis of MIC_{50} and MIC_{90} obtained on the Njo 2 strain, the HET turns out to be the most efficient with respective lower values of (1.73 and 23.34 ml/ml) against (4.76 and 26.03 ml/ml) for

the HEC. These EO could thus be exploited as biodegradable antifungal substances, likely to control white rot of bean.

Keywords

S. sclerotiorum, *T. vulgaris*, *C. Citratrus*, Essential Oils, Antifungal Activity, Inhibition

1. Introduction

The common bean, with its scientific name *Phaseolus vulgaris* L., is the most consumed dry vegetable in the world. In 2008, for a cultivated area of 26.47 million hectares in the temperate and tropical regions of America, its world production was estimated at 20.4 million tons for, Africa and Asia [1]. In Central, Eastern and Southern Africa, dry beans are the most important grain legume not only in terms of area cultivated but also in terms of consumption [2]. In Africa, small-scale farmers grow about 4 million hectares of beans each year. These crops are used to feed more than 100 million Africans [3]. Dry beans, also known as the "meat of the poor", are an alternative source of protein, carbohydrates, fiber, vitamins, and minerals in tropical and subtropical areas [4]. In Cameroon, the Western Highlands constitute the main common bean production basin. It includes both the Western and Northwestern regions with more than 90% of national production [5]; yields of local and exotic varieties are not clearly delineated.

All rural populations in the Highlands of West Cameroon grow several varieties of common bean for food and commercial purposes, with yields around 1.5 $t \cdot ha^{-1}$ [6]. Given the importance of common bean in several aspects, its national and international demand is increasing year after year although its production remains low due to losses caused by diseases and pests.

The cultivation and conservation of common bean is faced with various phytosanitary problems related to biotic constraints such as fungi, insect pests and rodents on the one hand and abiotic constraints such as temperature and humidity on the other hand [7]. Fungal attacks on *P. vulgaris* L. crops cause considerable production losses and damage in humid tropical biotopes. In Cameroon, the species *Sclerotinia sclerotiorum* is the main cause of white rot of the common bean crop in the field. This pathogen causes white rot in about 408 plant species (including *P. vulagaris* L.) of about 275 different varieties [8]. Despite much work to control this pathogen, it continues to be a major cause of yield loss. The use of synthetic fungicides is one of the most effective methods of controlling this type of pathogen [9]. However, good quality synthetic products are rare and expensive in rural areas; they very often have a harmful residual effect on human health and the biocenosis; they disturb ecological balances and pollute the environment [10] [11] [12].

Currently, various studies focus on the use of plants with fungicidal and insecticidal properties against diseases and insect pests of crops [13] [14] [15]. To this end, several works have shown and continue to show the antifungal potentialities of plant essential oils on the *in vitro* and *in vivo* growth of some fungi. Notably, the work of [16] on the *in vitro* inhibition of the growth of fungi such as *Rhizoctonia solani* J. G. Kuhn, *S. sclerotiorum* (Lib.) de Bary by the essential oils (EOs) of *Vitex agnus-castus* L. and *Myrtus communis* L; The use of *Eucalyptus storigeriana* EO on *Alternaria solani* [17] and the use of *Commiphora molmol* EO on the reduction of mycelial growth of *Aspergillus flavus*, *Fusarium oxysporum* and *A. alternata* [18]. The objective of this work is to control the mycelial growth of three strains of *S. sclerotiorum* fungi with the EO of two plants, namely *T. vulgaris* and *C. Citratrus*.

2. Material and Methods

2.1. Material

2.1.1. Plant Material

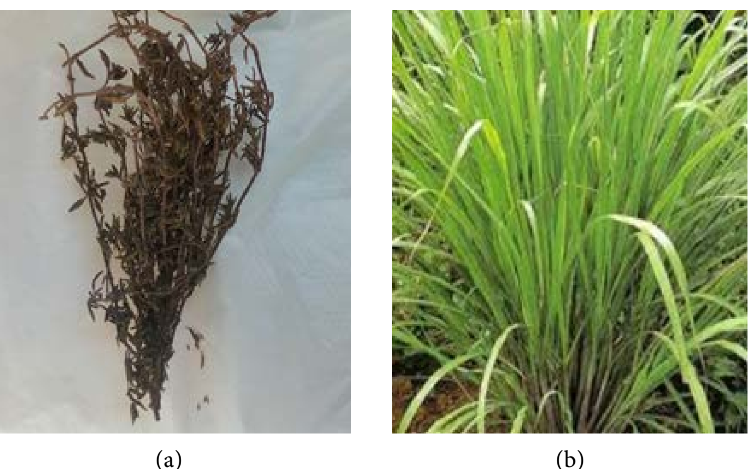
For the present work, two species of plants are used, namely thyme (*T. vulgaris*) and lemongrass (*C. citratrus*) (**Figure 1**). The leaves of *T. vulgaris* were purchased dry at the local medicinal plant market (Dakar market, Douala-Cameroon) while the leaves of *C. citratus* were harvested manually in the field and dried for 2 weeks in the air and out of the sun.

2.1.2. Chemical Material

The chemical material used is Banko-Plus 650 SC (Chlorothalonil at 550 g/L + Carbendazim at 100 g/L).

2.1.3. Fungal Material

The fungal material consisted of two strains of fungi from the Central Region, town of Biakoa (Bia 1 and Bia 2) and one strain from the Littoral Region, town of Njombé (Njo 2).



(a) (b)

Figure 1. Leaves of lant materials used: (a) *T. vulgaris*; (b) *C. Citratrus*.

2.2. Methods

2.2.1. Extraction Yield of Essential Oils

The extraction yields of lemongrass (HEC) and thyme (HET) essential oils were calculated in relation to the weights of plant material according to the following formula of [19].

$$R(\%) = \frac{Pe(\text{g})}{Pt(\text{g})} \times 100 \quad (1)$$

where:

R: yield

Pe: weight of the extract (essential oil)

Pt: total weight of dried plant material

2.2.2. *In Vitro* Evaluation of the Effect of Essential Oils on the Growth of Different Strains of *S. sclerotiorum*

1) Preparation of the culture medium

The preparation of the culture medium was done by taking 65 g of agar (Sabouraud Agar) [20] which are introduced into 1000 ml of sterile distilled water. The mixture is heated on a hot plate equipped with a magnetic stirrer for 10 minutes to homogenize the solution. The resulting solution is then autoclaved for 45 min at 120˚C and stored in the refrigerator for future use.

2) Isolation and purification of pathogen strains

Pure strains were isolated from infected bean leaves showing symptoms of the disease. The leaves were harvested in the field and then taken to the laboratory where they were washed several times with tap water, disinfected with a cotton soaked in 95% alcohol and then flame dried. The infected parts of the leaf (bearing the necroses) were removed using a scalpel and incubated in Petri dishes containing Sabouraud Agar medium. The Petri dish was then sealed with food film and incubated in a culture room at 22˚C - 24˚C under a 12 h photoperiod. Mycelia grew from the explant and reached sufficient growth after 5 days, ready for purification. Purification was carried out by successive subcultures of a fragment taken from the growth front of mycelium on Sabouraud Agar medium. This operation was repeated several times until pure cultures were obtained that will be kept in pill containers, containing sterile distilled water [21] [22]. Identification of the pathogen *S. sclerotiorum* was done through the observation of conidia on the microscope and an identification key [23] [24].

3) Essential oil extraction by hydrodistillation

The extraction of essential oils was carried out by hydro distillation using a hydro distillation apparatus type Clevenger [25]. The operation, which lasts 3 hours, consists in introducing 100 g of dried plant material (Thyme and Lemon grass) in a glass flask, then submerged the plants with a quantity of distilled water (1 L). Using a heating flask, the mixture is brought to a boil. The water vapors carry away the volatile essential oils, which are then condensed by a cold condenser (cooling coil) that transforms them into liquid. The essential oil is

lighter than water and floats to the surface. It is recovered, measured and weighed to determine the yield. The HET and HEC were dehydrated with sodium sulfate (Na_2SO_4) [26] and kept in a refrigerator at 4˚C and in the dark until analysis and future use [27].

4) Determination of the different doses of essential oils

To obtain the different doses of HEC and HET, volumes of 0.23, 0.45 and 0.9 ml were taken with a pipette and then, introduced respectively in 29.77, 29.55 and 29.1 ml of nutrient media cooled to 45˚C in order to obtain Sabouraud Agar media with respective concentrations of essential oils of 0.75, 1.5 and 3 ml/ml. For the synthetic fungicide, a stock solution was previously prepared by diluting 1 ml of Banko-plus 650 SC (550 g/L chlorothalonil + 100 g/L Carbendazim) in 200 ml of sterile distilled water. From this stock solution, a volume of 0.2 mL was pipetted into 29.8 mL of Sabouraud Agar nutrient medium. These culture media were poured into 90 mm Petri dishes. Agar discs of 6 mm diameter, bearing the mycelium of each isolate, were individually placed in the Petri dishes in the center of the different solidified culture media. The susceptibility of the fungus was assessed by measuring the radial growth of the mycelium every 24 h after two days of inoculation. The experiment lasted 08 days and was repeated 3 times.

5) Radial growth

The average growth (D) of *S. sclerotiorum* mycelium is determined by daily measurements of necrosis 48 h after incubation using a graduated scale. Each diameter is respectively measured on one of two lines forming a right angle through the center of the seeded explant (Figure 2). The percentages of inhibition (I %) of the different doses of EO and of the positive control are calculated after 7 to 9 days of fungal growth.

Mean radial growth and percent inhibition are calculated using the following formulas: [28].

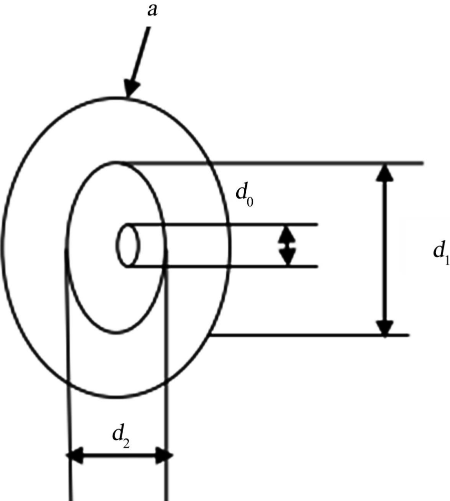


Figure 2. Diagram indicating the method of measuring the radius growth of pathogens.

$$D = \frac{d_1 + d_2}{2} - d_0 \quad (2)$$

where:

d_0 = diameter of the explant;

d_1 and d_2 = perpendicular diameters of the pathogen;

D = mean radial diameter.

$$I(\%) = \frac{\text{Dt0}(\text{mm}) - \text{Dxi}(\text{mm})}{\text{Dt0}(\text{mm})} \times 100 \quad (3)$$

where:

$I(\%)$ = percent inhibition;

Dt0 = average diameter of necrosis in the negative control;

Dxi = average diameter of necrosis in the HE or positive control.

6) Evaluation of fungicidal or fungistatic activities of essential oils

After incubation, the treatments in which the growth of the fungus was completely inhibited are counted and the explants are transferred to a new culture medium without EO. The extract is described as fungistatic if mycelial growth restarted and as fungicidal if not [29] [30].

7) Determination of minimum inhibitory concentrations

The values of the different inhibition percentages were used to determine the minimum inhibitory concentrations from the formula of [28]. From the linear regression equation between the neperian logarithms of the concentrations on the x-axis and the growth inhibition percentages on the *y*-axis, the concentrations reducing growth by 50% and 90% are determined [31].

8) Correlations between concentration and inhibition

The correlation between concentration and percentage inhibition was determined from the equation $y = ax + b$ with x = concentration, and y = percentage inhibition. In this case, if $a < 0$, the slope is negative; if $a > 0$, then the slope is positive; if r is between 0.8 and 1, then the correlation is perfect and positive; if r is between −0.8 and −1 then the correlation is perfect and negative; if r < 0.8 then the correlation is positive but imperfect; if r > −0.8 then the correlation is negative but imperfect [32].

2.3. Data Analysis

Data used were subjected to one-way analysis of variance (ANOVA) using SPSS 20.0 software. The multiple comparisons of means were performed using the Last significant difference (LSD) test at $p < 0.05$ to separate them when the analysis of variance was significant. The graphs were presented and the average grouping tables were produced in the Excel 2013 spreadsheet.

3. Results

3.1. Extraction Yield of Essential Oils

The two extractions having been made by hydrodistillation, yields of 1.5% ±

0.25% and 0.5% ± 0.1% were obtained with *C. citratus* and *T. vuglaris* respectively (Table 1).

3.2. Effect of Essential Oils on the Growth of Different Strains of *S. sclerotiorum*

3.2.1. Virulence of the Strains Used

The data in (Table 2) show the growth rate of the three strains of *S. sclerotiorum* used in this work regardless of the extracts and concentrations used. It appears that the Njo 2 and Bia 1 strains are the most virulent during 8 days of culture with respective radial growth diameters of 7.12 and 5.95 cm against 4.88 cm for the Bia 2 strain which thus proves to be the least virulent during the 8 days of culture.

3.2.2. Effects of Lemongrass Essential Oils (HEC) on the Growth of Different Strains

The results on the effect of HEC on the radial growth of the different strains of *S. sclerotiorum* tested show a considerable inhibition of the said radial growth at the different concentrations. A significant difference was observed between these 3 concentrations (C1, C2 and C3) which show different behaviors at the 5% threshold according to the LSD test. However, the Njo 2 strain proved to be more resistant to the different concentrations of HEC because, after 8 days of treatment, a high mycelial growth was observed at concentrations C1 = 0.75 ml/ml and C2 = 1.5 ml/ml, *i.e.* 7.12 and 7.07 cm respectively; these results are

Table 1. Extraction yield (%) and characteristics of lemongrass and Thyme Eos.

Leaves	Type of extraction	Solvent	Yield (%)	Color
C. citratus	Hydro distillation	water	1.5 ± 0.25	Yellow-orange
T. vuglaris	Hydro distillation	water	0.5 ± 0.1	Yellow-orange

Table 2. Radial growth of *S. sclerotiorum* strains (cm), whatever the extracts and concentrations used.

Period \ Strains	Bia 1	Bia 2	Njo 2
Day 2	1.10 a	0.88 b	1.17 a
Day 3	1.84 b	1.43 c	2.12 a
Day 4	2.35 b	2.06 c	3.15 a
Day 5	3.94 b	2.97 c	4.10 a
Day 6	4.26 b	3.58 c	5.51 a
Day 7	5.41 b	4.11 c	6.23 a
Day 8	5.95 b	4.88 c	7.12 a

*values of the same line followed by different letters are significantly different for $p < 0.05$ (LSD test).

not significantly different from the one observed in the control treatment (T− = 0 ml/ml) which showed a growth of 7.3 cm at the same time. On the other hand, the concentration C3 = 3 ml/ml showed a considerable inhibition (3.75 cm) which is statistically different from the previous three (**Figure 3**). The results also show in general that the strains of the Bia 1 and Bia 2 Center have almost the same behavior towards HEC.

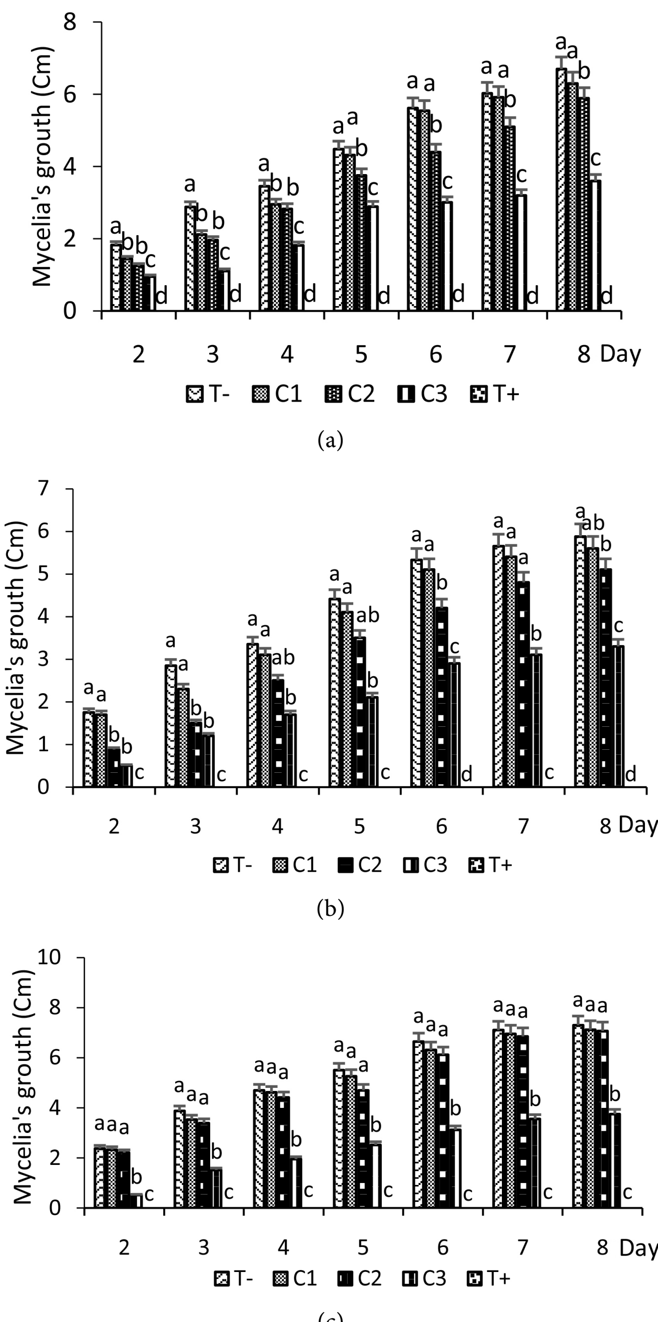


Figure 3. Effects of HEC on different strains of *S. sclerotirum* at different concentrations: (a) Bia 1, (b) Bia 2, (c) Njo 2. *Values for the same day with different letters are significantly different at $p < 0.05$ (LSD test).

3.2.3. Effect of Essential Oils of Thyme (HET) on the Growth of Strains

The HET presented a very high antifungal property with, in most cases, a percentage of inhibition of the order of 100%. A total inhibition of the growth of Bia 1 and Bia 2 strains and this is at all concentrations (C1, C2 and C3) was observed. The Njo 2 strain showed a slight resistance to HET at concentrations C1 = 0.75 ml/ml and C2 = 1.5 ml/ml, with radial growths of 7.12 and 6.5 cm respectively after 8 days of treatment. This strain, on the other hand, underwent a total inhibition of its growth at the concentration C3 = 3 ml/ml (Figure 4).

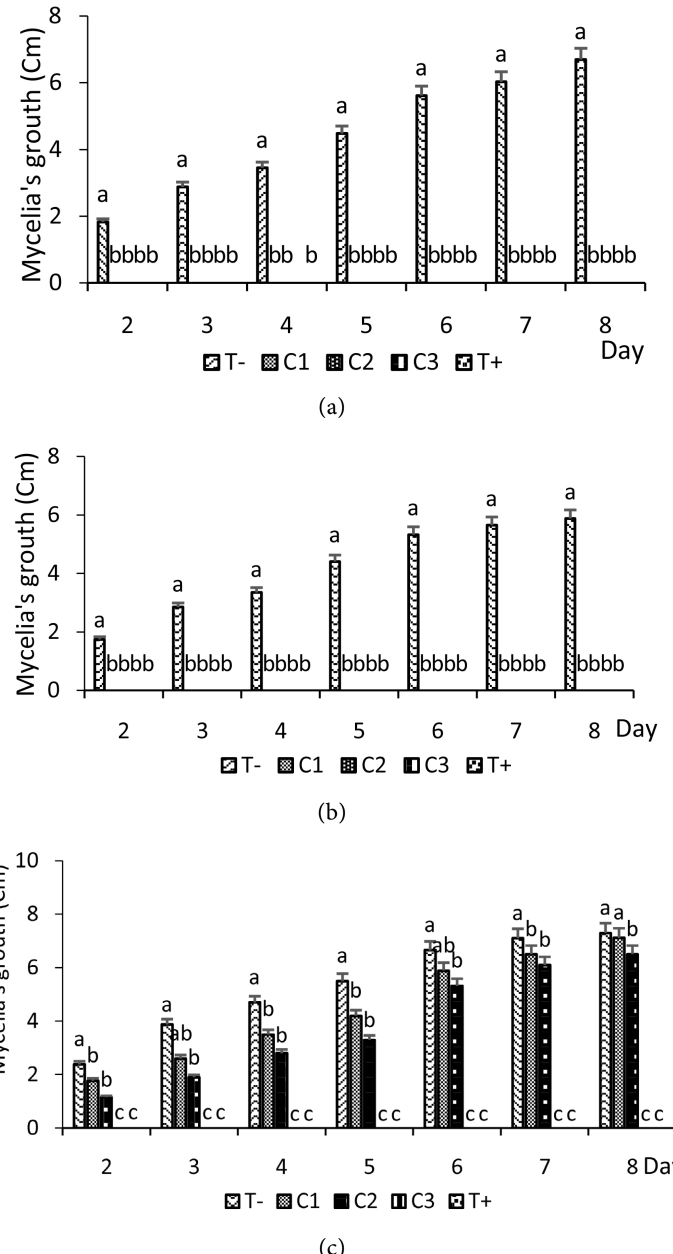


Figure 4. Effects of HET on different *S. sclerotirum* strains at different concentrations: (a) Bia 1, (b) Bia 2, (c) Njo 2. *Values of the same day with different letters are significantly different at $p < 0.05$ (LSD test).

The effect of HET evaluated on the Littoral strain (Njo 2), showed a significant repression of the fungus development. Compared to the control (0 ml/ml), the different concentrations used (C1: 0.75 ml/ml; C2: 1.5 ml/ml and C3: 3 ml/ml) showed significant inhibition of mycelial growth of the *S. sclerotirum* strain at each observation period. The inhibition here is proportional to the different concentrations, as it increases with EO concentration (**Figure 5**).

The results of the inhibition percentages obtained after 5 days of treatments show that the different essential oils significantly inhibited the mycelial growth of the strains at the different concentrations tested. A significant difference on the efficacy of the different concentrations within the same EO was observed for the three tested strains. As an illustration, a significant difference was observed between the percentages of inhibition obtained at the concentrations C1, C2 and C3 tested on the Njo 2 strain with the EO; we have respectively 9.82%; 20.63% and 54.18% (**Table 3**). Moreover, between the two HE, the results obtained show that HET are more effective than HEC. A significant difference is observed between these two EO when comparing the behavior of the same concentration on the same strain. Taking as an example the Bia 1 strain, a significant difference at the different concentrations (C1, C2 and C3) is observed: HEC (3.57%; 14.55% and 16.29%) versus HET (100; 100 and 100%) respectively (**Table 3**).

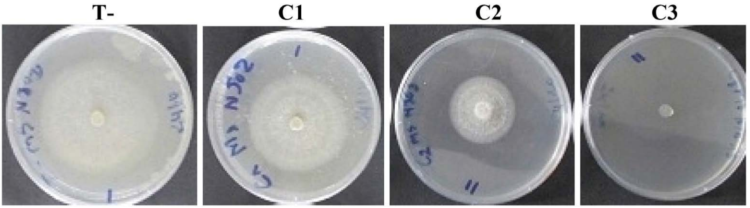


Figure 5. Mycelial growth of the Njo 2 strain in the HET treatment, 4 days after seeding.

Table 3. Percentage inhibition of EH on mycelial growth 5 days after treatments.

Strains	Concentrations	Percentage of inhibition (%)	
		HEC	HET
Bia 1	C1 (0.75 ml/ml)	3.57 ± 1.3 aA1	100 ± 0.0 aB2
	C2 (1.5 ml/ml)	14.55 ± 2.2 bA1	100 ± 0.0 aB2
	C3 (3 ml/ml)	16.29 ± 1.6 bA1	100 ± 0.0 aA2
Bia 2	C1 (0.75 ml/ml)	7.03 ± 0.7 aAB1	100 ± 0.0 aB2
	C2 (1.5 ml/ml)	15.17 ± 3.3 bA1	100 ± 0.0 aB2
	C3 (3 ml/ml)	40.0 ± 2.1 cB1	100 ± 0.0 aA2
Njo 2	C1 (0.75 ml/ml)	9.82 ± 1.1 aB1	35.49 ± 1.1 aA2
	C2 (1.5 ml/ml)	20.63 ± 1.2 bB1	52.38 ± 1.4 abA2
	C3 (3 ml/ml)	54.18 ± 0.8 cC1	100 ± 0.0 cA2

*values in the same row and column followed by different letters and numbers are significantly different for $p < 0.05$ (LSD test).

The triplet (a, b and c) was used to compare in the same EO and for the same strain, the behavior of the different concentrations; the triplet (a, b, c) was used to compare in the same EO and for the different strains, the behavior of the same concentration and, the doublet (1, 2) was used to compare between the different EO and for the same strain, the behavior of the same concentration.

3.2.4. Fungicidal/Fungistatic Properties of Extracts

For the fungicidal/fungistatic test conducted on the different strains of S. sclerotiorum, only the concentrations of essential oils that completely inhibited mycelial growth are mentioned. The HECs, at all concentrations tested did not totally inhibit mycelial growth; therefore, this test could not be performed for these extracts. With the HETs, a fungistatic activity was observed at concentration C1 = 0.75 ml/ml in the Bia 1 and Bia 2 strains, while concentrations C2 = 1.5 ml/ml and C3 = 3 ml/ml presented a fungicidal activity on these same strains. Njo 2 strain had complete inhibition of mycelial growth only at its C3 concentration for fungicidal activity with HET (**Table 4**).

3.2.5. Correlation Tests between Concentrations and Inhibition Percentages

The purpose of this test was to see if there is a linear relationship between the increase in mycelial growth inhibition of the pathogen and the different concentrations of the essential oils. The regression equations obtained with HEC showed increasing linear relationships between concentrations and inhibition percentages. All strains tested, showed regression lines with positive slopes: $y = 20.841x - 7.2933$; $y = 7.4058x + 72.747$ and $y = 23.594x + 13.173$, respectively for the strains Bia 1, Bia 2, and Njo 2 (**Figure 6**). For these three strains, the correlation coefficient values: r = +0.93, r = +0.87, and r = +0.87 were obtained respectively (**Table 5**).

Regarding HET, the regression equations obtained also revealed variable behaviors of the different strains used towards this extract. A positive slope was obtained for one of the three strains tested (**Figure 7**); this was the Njo 2 strain with a slope of $y = 15.362x + 41.533$ and a correlation coefficient of r = 0.97 (**Table 6**).

Table 4. Fungicidal or fungistatic activity of different essential oils.

Strains	Concentrations	HET
Bia 1	C1	Fungistatic
	C2	Fungicidal
	C3	Foungicidal
Bia 2	C1	Fungistatic
	C2	Fungicidal
	C3	Fungicidal
Njo 2	C3	Fungicidal

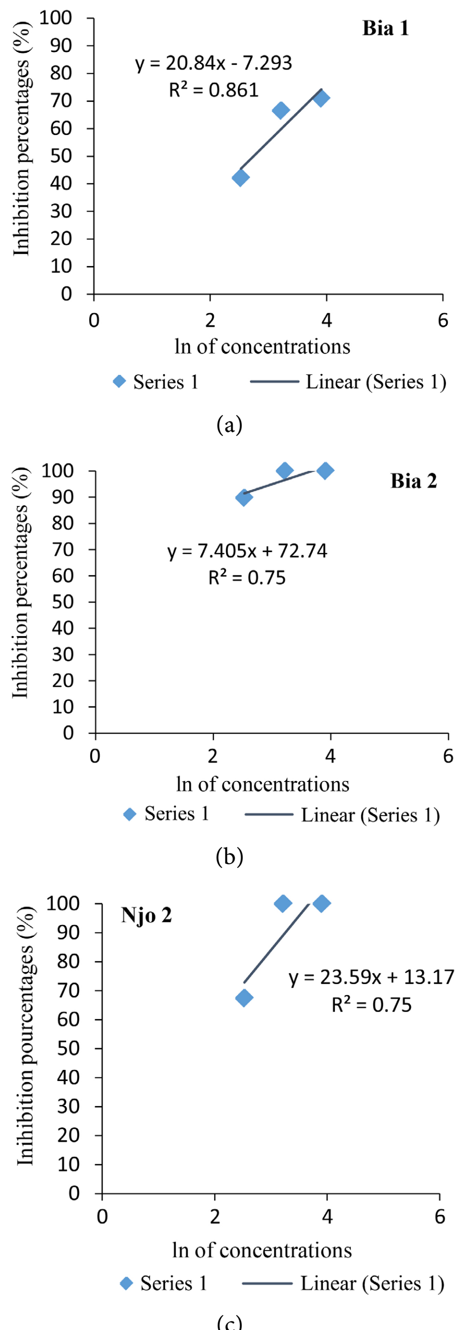


Figure 6. Regression lines on the growth of the different strains after treatment with HEC.

Table 5. Correlation between percentage of inhibition and concentrations of essential oils on strains tested with HEC.

Strains	Correlation coefficient*	Observation
Bia 1	+0.93	Strongly correlated
Bia 2	+0.87	Strongly correlated
Njo 2	+0.87	Strongly correlated

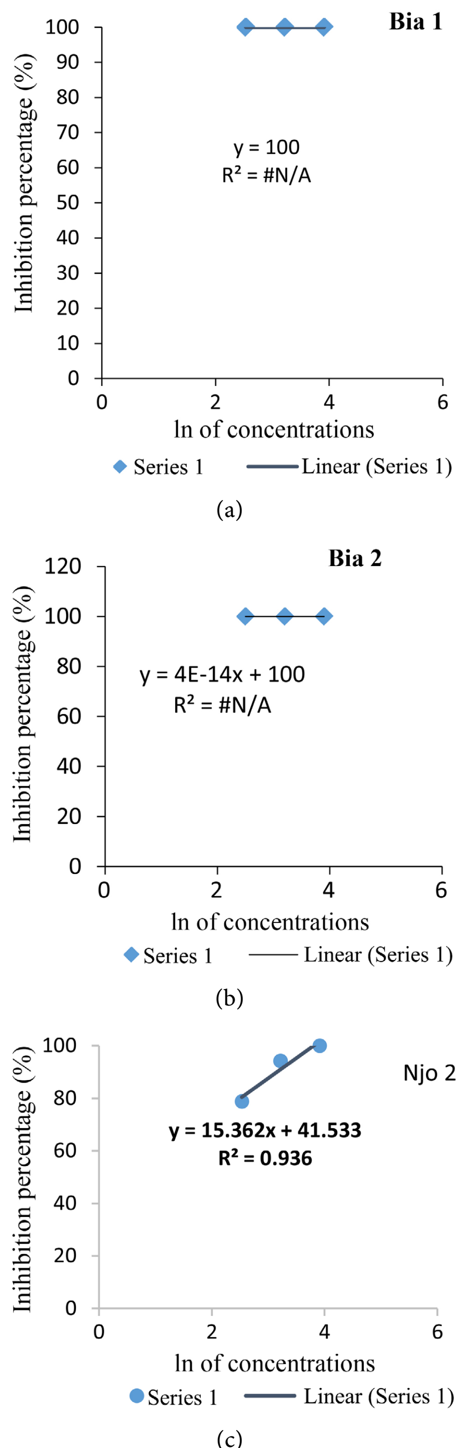


Figure 7. Regression lines on the growth of the different strains after HET treatments.

Table 6. Correlation between percentage of inhibition and concentrations of essential oils on the strains tested with the HET.

Strains	Correlation coefficient®	Observation
Njo 2	+0.97	Strongly correlated

For all these strains tested, the correlation between different concentrations and percentage of inhibition is therefore perfect and positive because, the correlation coefficient r being greater than 0.7.

3.2.6. Minimum Inhibitory Concentrations MIC_{50} and MIC_{90} on Some Strains Used

From the regression lines obtained after the correlation tests, the concentrations inhibiting by 50% (MIC_{50}) and 90% (MIC_{90}) the growth of the different pathogen strains were determined.

The lowest inhibitory concentrations were obtained with HET, justifying their greater efficacy compared to HEC. The MIC_{50} obtained with HEC on all three strains tested ranged from 4.76 ml/ml for the Njo 2 strain to 15.71×10^6 ml/ml for the Bia 2 strain. The MIC_{90} vary from 20.05 m/ml in the Njo 2 strain to 3.47 $\times 10^9$ ml/ml in the Bia 2 strain for the same treatment. On the other hand, for the treatment of these same three strains with HET, the MIC_{50} and MIC_{90} could only be defined for the Njo 2 strain where they were respectively 1.73 ml/ml and 23.34 ml/ml. On the Bia 1 and Bia 2 strains, growth inhibition was total and therefore it was not possible to determine them (Table 7).

4. Discussion

The yields obtained after extraction of the essential oils of the two investigated plants (1.5% for HEC and 0.5% for HET) show that this parameter varies from one plant species to another. The yield of HEC obtained in this study is higher than that obtained by [33] which is 0.7% but, lower than those obtained on the same plant species by [34] [35] which are around 4.31%. The difference in yield of these two plants can be explained by the influence of some extrinsic factors such as the geographical origin of the raw material; the cultural conditions such as sowing date, harvesting date, phytosanitary treatments, fertilizer use, and harvesting techniques [36] [37]. Concerning HET, the results of the extraction yield are in agreement with those obtained on the same plant by [38] who obtained a yield of 0.65%.

Generally speaking, the results of this work show a significantly positive effect of essential oils, especially HET, in the various tests carried out on white rot of beans in the laboratory. The different strains tested showed different behaviors

Table 7. MIC_{50} and MIC_{90} of mycelial growth of the different strains with the tested extracts (ml/ml).

Strains	HEC		HET	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Bia 1	15.64	106.7	*	*
Bia 2	15.71×10^6	3.47×10^9	*	*
Njo 2	4.76	26.05	1.73	23.34

*Represents values that are not set to be zero statistically.

from each other. The Littoral strain (Njo 2) was the most aggressive (most virulent) with a necrosis growth of 7.2 cm at day 8. Such results were obtained by [39] and [40] in studies based on the characterization of some fungal strains from the Central, Southern and Littoral regions.

The fact of obtaining strains more resistant (Njo 2) to EO than others would be due to the non-specificity that these different strains would present at the membrane level. Indeed, antifungals can be contact (the antifungal acts at the membrane of the fungus) or systemic: the antifungal acts inside the cell [41]. In either case, specific membrane or intracellular receptors may be essential for the expression of the antifungal's biological activity [42].

HET and HEC significantly inhibited the growth of the different fungal strains tested. However, inhibition percentages of the order of 100% were obtained with HET at all concentrations on the strains Bia 1 and Bia 2 and, at the concentration C3 = 3 ml/ml on the strain Njo 1. The work of [35] showed the effectiveness of some essential oils including those of *C. citratus* L. of *C. nardus* L. and *Eucalyptus saligna* on the weathering mycoflora of *Vignia unguiculata* L. in South-Benin. Similarly, [43] showed the efficacy of *C. schoenanthus* EO with inhibitions ranging from 26% to 100% at doses of 1%, 5%, 10%, 50% and 100% *in vitro* conditions.

These results obtained showing considerable inhibition of mycelial growth (100%) at different concentrations of EO are in line with those of [38] who, comparing in their work the antifungal activity of three essential oils (*T. vulgaris*, *C. limonum* and *Mentha spicata*), on the growth of some dermatophytes had a significant effect with the essential oils of *T. vulgaris* at concentrations greater than or equal to 2% against equally significant effects but, at high concentrations of 4% and 10% respectively for the essential oils of *M. spicata* and *C. limonum*. According to [44], the antifungal efficacy of EO is related to an active substance called thymol. [45] have shown that HET exhibited a high content of Thymol (41.04%) as major compound over 14 identified components by GC-MS analysis followed by 1,8-Cineole (14.26%), γ-Terpinene (12.06%), p-Cymene (10.50%) and α-Terpinene (9.22%).

The MIC_{50} and MIC_{90} of the different EO were determined with the different strains tested. An important variability was observed; the HET presenting the lowest values, which justifies their effectiveness and consequently their fungicidal potential. This variability could be explained by the fact that phytopathogenic fungi do not react in the same way to biopesticides. Such results were obtained by [46] who showed that phytopathogenic fungi act differently in the presence of biopesticides. These results highlighting the higher efficacy of HET via low MIC (50 and 90) (1.73 and 23.34 ml/ml) compared to HEC: (4.76 and 26.05 ml/ml) respectively, are in line with those obtained by [46] who obtained high inhibition percentages on cowpea spoilage mycoflora with MIC values between 0.05 and 1 µl/ml. In addition, [47] also obtained similar results on the radial growth of some fungi with low MIC 50, 90 and 95 of the aqueous extracts of *Thevetia pe-*

ruviana, *i.e.* 2.9; 4.3 and 4.48 ml/ml.

The effectiveness of Banko-Plus 650 SC has been demonstrated once again by completely inhibiting the mycelial growth of the different strains of *S. sclerotiorum* tested. This product is known to have a direct and toxic action against some fungi in general and *S. sclerotiorum* in particular through a persistence of its active principles, namely Chlorothalonil and Carbendazim.

5. Conclusion

The objective of this work was to evaluate the antifungal activity of essential oils of two aromatic plants against three strains of *S. sclerotiorum* responsible for white rot of bean. The different essential oils used showed significantly high efficacy on the reduction of mycelial growth with a particularly satisfactory behavior obtained with HET (100% inhibition) at all concentrations. At different concentrations and according to the two EO used, the Njo 2 strain proved to be the most aggressive while the Bia 2 strain was more sensitive to the different treatments. These different extracts could be, depending on the efficiencies presented, exploited as biodegradable antifungal substances able to control white rot of beans.

Acknowledgements

To the colleagues for their time and scientific quality for the writing of this work as well as the volunteers of the laboratory.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Wortmann, C.S. (2006) *Phaseolus vulgaris* L. (Common Bean): Plant Ressources of Tropical Africa/Ressources Végétales de l'Afrique tropicale (Prota 1). CIAT: Cali, Colombia.

[2] Anonyme (2005) Application de la biotechnologie à la lutte contre les maladies du haricot. N° 27; Le CIAT en Afrique.

[3] Pabra (2006) Nutrition et santé: La vision de PABRA. Alliance Panafricaine de Recherche sur le Haricot, 3: 2.

[4] Wortmann, C.S., Kirkby, R.A., Elude, C.A. and Allen, D.J. (1998) Atlas of Common Bean (*Phaseolus vulgaris* L.) Production in Africa (Prota 2). CIAT: Cali, Colombia.

[5] Anonyme (2010) Annuaire des Statistiques du Secteur Agricol du Cameroun. https://www.minader.cm/documents-destatistiques-de-la-direction-des-études-etstatistiques-agricoles

[6] Pamo, T.E., Boukila, B., Tankou, M.C., Tendonkeng, F., Kana, J.-R. and Loudjom, D.A. (2005) Effet de différentes sources d'azote sur la croissance et le rendement du haricot commun (*Phaseolus vulgaris* L.) à l'Ouest Cameroun. *Cameroon Journal of Experimental Biology*, **1**, 1-7. https://doi.org/10.4314/cajeb.v1i1.37930

[7] Diby, Y.K.S., Tahiri, Y.A., Akpesse, A.A.M. and Tra Bi, C.S. (2015) Graine: Haricot. In: *Agriculture en Afrique Tropical*, DGCI, Bruxelles, 337-355.

[8] Boland, G.J. and Hall, R. (1994) Index of Plant Hosts of *Sclerotinia sclerotiorum. Canadian Journal of Plant Pathology*, **16**, 93-108. https://doi.org/10.1080/07060669409500766

[9] Haubruge, E., Shiffers, B., Gabriel, E. and Verstraeten, C. (1988) Etude de la relation dose-efficacité de six insecticides à l'égard de *Sitophilus granarius* L., *S. oryzae* L., *S. zeamais* Mots. (Coleoptera, Curculionidae). Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent. 53/2b, 719-726.

[10] Condor-Golec, A.F. (2007) Effect of Neem (*Azadirachta indica* A. Juss) Insecticides on Parasitoids. *Revista Peruana de Biología*, **14**, 69-74. https://doi.org/10.15381/rpb.v14i1.1762

[11] Regnault-Roger, C. (2007) Biopesticides: Une alternative aux pesticides conventionnels? *Mer*, *Littoral*, *Lacs et Cours d'eau*, **73**, 60-64.

[12] Chemat, F., Vian, A.M. and Cravotto, G. (2012) Green Extraction of Natural Products: Concept and Principles. *International Journal of Molecular Sciences*, **13**, 8615-8627. https://doi.org/10.3390/ijms13078615

[13] Doumbouya, M., Abo, K., Lepengue, A.N., Camara, B., Kanko, K., Aidara, D. and Kone, D. (2012) Comparing *in Vitro* Activities of Two Synthetic Fungicides and Two Essential Oils on Soil Fungi of Vegetable Crops in Côte d'Ivoire. *Journal of Applied Biosciences*, **50**, 1-13.

[14] Messaoundene, H. and Mouhou, N. (2017) Etude de la toxicité des huiles essentielles contre les ravageurs des denrées stockées. Etude de la toxicité des huiles essentielles contre les ravageurs des denrées stockées. Mémoire de Doctorat, Faculté des Sciences de la Nature et la Vie, Université Abderrahmane MIR-Bejaia, Algérie, 35 p.

[15] Lipi, P., Agindotan, B.O. and Burrows, M.E. (2021) Antifungal Activity of Plant-Derived Essantial Oils on Pathogens of Pulse Crops. *Plant Disease*, **105**, 1692-1701. https://doi.org/10.1094/PDIS-06-20-1401-RE

[16] Yilar, M., Bayan, Y. and Onaran, A. (2016) Chemical Composition and Antifungal Effects of *Vitex agnus-castus* L. and *Myrtus communis* L. Plants. *Notulae Botanicae Horti Agrobotanici Cluj Napoca*, **44**, 466-471. https://doi.org/10.15835/nbha44210399

[17] Tomazini, E.Z., Pauletti, G.F., Ribeiro, R.T.S., Moura, S. and Schwambach, J. (2017) *In Vitro* and *in Vivo* Activity of Essential Oils Extracted from *Eucalyptus staigeriana*, *Eucalyptus globulus* and *Cinnamomum camphora* against *Alternaria solani* Sorauer Causing Early Blight in Tomato. *Scientia Horticulturae*, **223**, 72-77. https://doi.org/10.1016/j.scienta.2017.04.033

[18] Perveen, K., Bokhari, N.A., Siddique, I. and AlRashid, S.A.I. (2018) Antifungal Activity of Essential Oil of Commiphoramolmol Oleo Gum Resin, *Journal of Essential Oil Bearing Plants*, **21**, 667-673. https://doi.org/10.1080/0972060X.2018.1492975

[19] Ngoh Dooh, J.P., Ambang, Z., Ndongo, B., Heu, A. and Kuate, T.W. (2014) Effect of Extracts of *Thevetia peruviana* (Pers.) K. Schum on Development of *Phytophthora megakarya* Causal Agent of Black Pod Disease of Cocoa. *Journal of Applied Biosciences*, **77**, 6564-6574. https://doi.org/10.4314/jab.v77i0.11

[20] Sabouraud, R. (1892) Contribution to the Study of Human Trichophytia. 1061-1087. https://www.babordnum.fr/files/original/8836eb284e7920ac5d7f3f9312da8068.pdf

[21] Ondo, A.S. (2006) Charactérisation de quelques isolats de *P. megakarya* agent causal de la pourriture brune des cabosses de cacaoyer (*Theobroma cacao* L.). Mémoire de DEA, Université de Yaoundé I., Yaoundé, 58 p.

[22] Nyassé, S. (1992) Structure d'une population de *Phytophthora* spp. des cacaoyères camerounaises atteintes de pourriture brune. Mémoire de diplôme de Recherche Universitaire ENSAT, Toulouse, 43 p.

[23] Vaz, P.D.C. (1987) IMP Description of Fungi and Bacteria. 92, sheet 916.

[24] Hsieh, W.H. and Goh, T.K. (1990) Cercospora and Similar Fungi from Taiwan. Maw Chang Book Company, Taipei. https://www.bcrc.firdi.org.tw/fungi/fungal

[25] Oussou, K.R. (2009) Etude chimique et activités biologiques des huiles essentielles de sept plantes aromatiques de la pharmacopée ivoirienne. Thèse de Doctorat Unique, Laboratoire de Chimie Organique et Structurale, UFR SSMT, Université de Cocody-Abidjan, Abidjan, 241 p.

[26] Bajpai, V.K., Shukla, S. and Kang, S.C. (2008) Chemical Composition and Antifungal Activity of Essential Oil and Various Extract of *Silene armeria* L. *Bioresource Technology*, **99**, 8903-8908. https://doi.org/10.1016/j.biortech.2008.04.060

[27] Willem, J.P. (2004) Les huiles essentielles, Médecine d'avenir. Ed, Dauphin, Paris, 311.

[28] Singh, G., Padvay, R.K., Narayanam, C.S., Padmhurmeri, K.P. and Rao, G.P. (1993) Chemical and Fungitoxic Investigations on the Essential Oil of Citrus Sinensis. *Pers. Z. dentshe zeeits halft fur pflanzenfrankenen und flanzenschustz*, **100**, 69-74.

[29] Kishore, N., Mishra, A.K. and Cham Souria, J.P.N. (1993) Fungitoxicity of Essential Oils against Dermatophytes. *Myc*, **36**, 211-215. https://doi.org/10.1111/j.1439-0507.1993.tb00753.x

[30] Pandey, D.K., Chandra, H. and Tripathi, N.N. (1982) Volatile Fungitoxicity Activity in Higher Plants Special Reference to That of *Callistemun lanceolatus* DC. *Phytopathology*, **105**, 175-182. https://doi.org/10.1111/j.1439-0434.1982.tb00675.x

[31] Dohou, N., Yamni, K., Badoc, A. and Douira, A. (2004) Activité antifongique d'extraits de *Thymelaea lythroides* sur trois champignons pathogènes du riz. *Bulletin des Sociétésde Pharmacie de Bordeaux*, **143**, 31-38.

[32] Heu, A. (2013) Effet des extraits de graines de *Thevetia peruviana* sur la germination des spores de *Phytophthora megakarya* et de *Colletotrichum gloeosporioides in Vitro*. Mémoire de Master, Université de Yaoundé I, Yaoundé, 52 p.

[33] Kanko, C. (2010) Contribution à l'étude phytochimique de plantes médicales et aromatiques de Côte d'Ivoire. Activité analgésique et antiinflammatoire de stérols isolés de l'écorce de Parkia biglobosa. Thèse de doctorat, Université de Cocody-Abidjan, Abidjan, 224 p.

[34] Konfo, C., Ahoussi-Dahouenon, E., Sessou, P., Yehouenou, B., Djenontin, S., de Souza, C. and Sohounhloue, D. (2012) Stabilization of Local Drink "Tchakpalo" Produced in Benin by Addition of Essential Oil Extracted from Fresh Leaves of *Cymbopogon citratus*. *International Research Journal of Biological Sciences*, **1**, 40-49.

[35] Kpatinvoh, B., Adjou Euloge, S., Dahouenon-Ahoussi, E., Konfo, T.R.C., Atrevi, B., Soumanou Mohamed, M. and Sohounhloue, D.C.K. (2017) Efficacité des huiles essentielles de trois plantes aromatiques contre la mycoflore d'altération du niébé collecté dans les magasins de vente du Sud-Bénin. *Journal of Applied Biosciences*, **109**, 10680-10687. https://doi.org/10.4314/jab.v109i1.12

[36] Benini, C. (2007) Contribution à l'étude de la diversification de la production

d'huiles essentielles aux Comores. Mémoire d'ingéniorat. Université Gembloux, Gembloux, 109 p.

[37] Aprotosoaie, A.C., Spac, A.D., Hancianu, M., Miron, A., Tanasescu, V.F., Dorneanu, V. and Stanescu, U. (2010) The Chemical Profile of Essential Oils Obtained from Fennel Fruits (*Foeniculum vulgare* Mill.). *Farmacia*, **58**, 46-54.

[38] Rachid, I., Abdeslam, L. and Khadija, M. (2014) Study of the Antifungal Activity of Essential Oils of Three Moroccan Aromatic Plants. *International Journal of Innovation Science and Research*, **12**, 499-505.

[39] Ngoh Dooh, J.P., Ambang, Z., Tih Ewola, A., Heu, A., Kosma, P., Maho yalen, E.J. and Ghogomu Tih, R. (2014) Screening and the Effect of Extracts of *Thevetia peruviana* on the Development of *Colletotrichum gloeosporioides*, Causal Agent of Cassava Anthracnose Disease. *E3 Journal of Agricultural Research and Development*, **4**, 54-65.

[40] Nchare, S.F. (2013) Évaluation du potentiel antifongique des extraits de graines de *Jatropha curcas* sur le développement *in Vitro* des souches de *Phytophthora megakarya.* Mémoire de Master, Université de Yaoundé I, Yaoundé, 52 p.

[41] Zacchino, S., Rodriguez, G., Santecchia, C., Pezzenati, G., Giannini, F. and Enriz, R. (1998) *In Vitro* Studies on Mode of Action of Antifungal 804'-Neolignans Occuring in Certain Species of Virola and Related Genera of Myristicaceae. *Journal of Ethnopharmacology*, **62**, 35-41. https://doi.org/10.1016/S0378-8741(98)00056-7

[42] Mboussi, S., Ambang, Z., Ndogho, P., NgohDooh, J. and Manga Essouma, F. (2016) *In Vitro* Antifungal Potential of Aqueous Seeds Extracts of *Azadirachta indica* and *Thevetia peruviana* against *Phytophthora megakarya* in Cameroon. *Journal of Applied Life Sciences International*, **4**, 1-12. https://doi.org/10.9734/JALSI/2016/23710

[43] Sirima, A., Sereme, A., Sereme, D., Koïta, K., Nana, T.A. and Sawadogo, M. (2020) Effects of Four Essential Oils on Radial Mycelial Growth of an Isolate of *Alternaria* sp. in Burkina Faso. *International Journal of Biological and Chemical Sciences*, **14**, 762-771. https://doi.org/10.4314/ijbcs.v14i3.10

[44] Cimanga, K., Kambu, K. and Tona, L. (2002) Correlation between Chemical Composition and Antibacterial Activity of Essential Oils of Some Aromatic Medicinal Plants Growing in the Democratic Republic of Congo. *Journal Ethnopharmacology*, **79**, 213-220. https://doi.org/10.1016/S0378-8741(01)00384-1

[45] Aljabeili, S., Barakat, H. and Abdel-Rahman, A.H. (2018) Chemical Composition, Antibacterial and Antioxidant Activities of Thyme Essential Oil (*Thymus vulgaris*). *Food and Nutrition Sciences*, **9**, 433-446. https://doi.org/10.4236/fns.2018.95034

[46] Carlton, R., Watermann, R., Gray, A.I. and Deans, S.G. (1992) The Antifungal Activity of Leaf Gland Volatile Oil of Sweet Gale (*Myrica gale*) Myricaceae. *Chemaecology*, **3**, 55-59. https://doi.org/10.1007/BF01261457

[47] Mboussi, S.B., Heu, A., Kone Nsangou, A.N., Douanla Ajebe, M., Ntsomboh Ntsefong, G., Ngoh Dooh, J.P. and Ambang, Z. (2021) *In Vitro* Evaluation of the Fungicidal Potential of Aqueous and Methanolic Extracts of *Thevetia peruviana* on the Development of *Rigidoporus lignosus*, Causal Agent of White Root Rot of *Hevea brasiliensis*. *Biotechnology Journal International*, **25**, 1-13. https://doi.org/10.9734/bji/2021/v25i130127