



**Original Paper**

<http://ajol.info/index.php/ijbcs>

<http://indexmedicus.afro.who.int>

## Comparative efficacy of essential oils of three aromatic plants as alternatives in the control of *Phytophthora* spp., agent of cocoa tree (*Theobroma cacao* L.) black pod disease in Côte d'Ivoire

Klotioloma COULIBALY<sup>1\*</sup>, F. J. M. Koffi KASSI<sup>2</sup>, Adama OUATTARA<sup>1,3</sup>,  
GOGBE-Dibi B. F.<sup>1</sup>, P. Walet N'GUESSAN<sup>1</sup>, J.A. Kotaix ACKA<sup>1</sup>, M. Evelyne ASSI<sup>1</sup>,  
N. N'Dri KOUAME<sup>1</sup>, Brigitte GUIRAUD<sup>1</sup>, M. Gnion TAHI<sup>1</sup>, Daouda KONE<sup>2</sup>,  
Ibrahim KONATE<sup>3</sup> and F. Kouamé N'GUESSAN<sup>1</sup>

<sup>1</sup> National Center of Agronomic Research, PO Box 808, Divo (Côte d'Ivoire).

<sup>2</sup> Laboratory of Plant Physiology, Faculty of Biosciences, Félix Houphouët-Boigny University, 22 PO Box 582 Abidjan 22 (Côte d'Ivoire).

<sup>3</sup> Laboratory of Agrovalorization, Department of Biochemistry and Microbiology, Faculty of Agroforestry, Jean Lorougnon Guédé University, PO Box 150 Daloa (Côte d'Ivoire).

\* Corresponding author; E-mail: [coolklotiolo@yahoo.fr](mailto:coolklotiolo@yahoo.fr)

Received: 26-05-2021

Accepted: 21-07-2021

Published: 31-08-2021

### ABSTRACT

This study aimed at comparing the efficacy of essential oils from three aromatic plants species (*Cymbopogon citratus*, *Eucalyptus citriodora* and *Ocimum gratissimum*) *in vitro* and *in vivo* against two *Phytophthora* strains (*Phytophthora palmivora* and *P. megakarya*) in a view to developing new biofungicides for the control of cocoa tree black pod disease. Essential oils were applied at four different concentrations (0.5; 1; 3 and 5 mL/L) *in vitro* and *in vivo* on leaf discs of three cocoa clones (NA32, PA150 and SCA6) which susceptibility is known. As results, all three essential oils significantly ( $p < 0.05$ ) reduced *in vitro* mycelial growth of both *Phytophthora* strains and also reduced the leaf susceptibility to these strains. Inhibition rates *in vitro* of *Phytophthora* strains mycelial growth ranged from 83.80 to 100% for the essential oils of *Cymbopogon citratus* and *Ocimum gratissimum* species and from 17.7 to 100% for which of *Eucalyptus citriodora* species. In the biotests, the leaf susceptibility index of clone NA32 (susceptible) was reduced from 3.14 to 0.40. This correspond to reduction rates ranged from 64.87 to 86.70% of leaf susceptibility to both *Phytophthora* strains. Essential oils (HS and HO) from *Cymbopogon citratus* and *Ocimum gratissimum* species were most effective than which from *Eucalyptus citriodora* species. However, all three essential oils revealed antifungal properties. Therefore, they could be recommended as biofungicides for a sustainable control of cocoa tree black pod disease in Côte d'Ivoire. © 2021 International Formulae Group. All rights reserved.

**Keywords:** Efficacy; Essential oils; *Phytophthora*; Black pod disease; Biofungicides.

### INTRODUCTION

In Côte d'Ivoire, cocoa is the main export crop (ICCO, 2000). With a production of 1,964,000 tons in 2017-2018, Côte d'Ivoire

is the world's leading cocoa producer (ICCO, 2019). Cocoa farming occupies an area of over 2,000,000 hectares (6% of the national territory) and involves a farming population of

over 800,000 producers (CCC, 2014; Friedel et al., 2016). Cocoa generates more than 30% of export earnings and contributes more than 15% to the Gross Domestic Product (GDP); (CCC, 2014; Friedel et al., 2016). Despite this remarkable performance, cocoa farming remains subject to numerous production constraints (Assiri et al., 2012; Ploetz, 2016). Among these constraints, black pod disease is currently one of the main sources of productivity losses in Ivorian cocoa farms (Coulibaly et al., 2018). It has been on the rise in recent years in Côte d'Ivoire, mainly in the South-East, Centre-West and West zones with estimated losses of 35 to 50% (Coulibaly, 2014; Coulibaly et al., 2018).

Faced with the threat of black pod disease, synthetic fungicides are essentially used (Mpika et al., 2009; Pohe et al., 2013; Bailey et al., 2016). Under optimal conditions, their effectiveness is certain. However, agrochemical use can be problematic due to risk of pesticides residues in cocoa beans, intoxication and environmental pollution (Deberdt et al., 2008; Yapo et al., 2014; Gramlich et al., 2016). In response to problems associated with pesticides use as well as consumer desire for organic chocolate (Gramlich et al., 2017; Kareem, 2016), a sustainable management method, less costly and environmentally friendly must be developed (Ten Hoopen and Krauss, 2016; Ouattara et al., 2020). Thus, there has been increased interest for biological control through the use of aromatic plants with antifungal and antibacterial properties (Oxenhan et al., 2005; Jiménez-Reyes et al., 2019). Extracts and essential oils of various aromatic plants have demonstrated their effectiveness for management of many plant diseases (Camara et al., 2007; Doumbouya et al., 2012; Pohe and Agneroh, 2013; Kassi et al., 2014). Several essential oils from plant species *Azadirachta indica*, *Melaleuca alternifolia*, *Ocimum* sp., exhibited antifungal activities against various pathogens commonly encountered on crop products (Camara et al., 2010; Doumbouya et al., 2012; Muthukumar et al., 2016; Tiendrebeogo et al., 2017). This study aimed at comparing the efficacy of

essential oils of *Cymbopogon citratus*, *Eucalyptus citriodora* and *Ocimum gratissimum* species *in vitro* and *in vivo* against two *Phytophthora* strains (*P. palmivora* and *P. megakarya*) in a view to developing new biofungicides for the control of cocoa tree black pod disease.

## MATERIALS AND METHODS

### Materials

#### *Plant material*

Plant material consisted of cocoa trees leaves and those of aromatic plants. Cocoa leaves came from three clones, which susceptibility to black pod disease is known. These clones were NA32, PA150 and SCA6 respectively susceptible, moderately resistant and resistant to black pod disease. The clones were collected in plots of the reciprocal recurrent selection program's collection at National Agronomic Center Research (CNRA), Research station of Divo (Côte d'Ivoire). Aromatic plant leaves came from three species (*Cymbopogon citratus*, *Eucalyptus citriodora* and *Ocimum gratissimum*) known for their antimicrobial activities and collected in three regions: Abidjan (South-East), Divo (Centre-West) and Korhogo (North) of Côte d'Ivoire.

#### *Fungal material*

Fungal material consisted of two strains of *Phytophthora*: *Phytophthora palmivora* (Strain BL7.11.2) and *P. megakarya* (Strain 13P30.1). These strains were isolated from naturally affected cocoa pods by black pod disease (Mpika et al., 2009; Ouattara et al., 2020).

### Methods

#### *Isolation and culture of Phytophthora*

Isolation of *Phytophthora* was performed by taking a piece of cocoa pod from the growth front of the pathogen (Mpika et al., 2009). This piece was placed on a 1.5% agar water medium. On this medium, the mycelium grew in the absence of bacteria. A mycelial fragment was taken from the growth front and transferred to a pea extract agar medium (Huguenin and Boccas, 1971). Incubation was carried out at 26 °C for 4 to 5 days. To avoid

heterogeneity of zoospores, cloning of each strain by mono zoospore isolation was carried out according to the method described by Babacauh (1980).

#### **Extraction of essential oils**

The harvested leaves were previously dried at laboratory room temperature (25 °C) for 5 days followed by extraction of essential oils. These oils were obtained by steam entrainment performed with a Clevenger-type device for 3 hours (Oussou, 2009; Castro et al., 2010; Santos et al., 2013). This method is a classical distillation during which the plant material was immersed in water and the whole brought to boiling. The water vapor loaded with volatile substances condenses inside a refrigerant. The essences, which are less dense than water, were collected in flasks by simple decantation on the surface of the water. The flasks containing essential oils were covered with aluminum foil to preserve them from any negative effect of light. The essential oils were then stored in a refrigerator at a temperature of 12 °C (Oussou, 2009).

#### **In vitro activity of essential oils**

The antifungal activity of essential oils was evaluated *in vitro* and aimed to determine the minimum inhibitory concentration of these oils. The essential oils were aseptically incorporated at decreasing concentrations (0.5; 1; 3; 5 mL/L) into pea extract agar medium after autoclaving at 121 °C, 1 bar for 30 min (Camara et al., 2010; Degnon et al., 2013; Kouamé et al., 2015 ). These media were poured at a rate of 20 mL into 9 mm diameter Petri dishes. For each concentration of each essential oil and for each strain of *Phytophthora*, four Petri dishes were inoculated with a mycelial explant of 6 mm diameter. These explants were die-cut from the growth front of a 4-day-old non-sporulating cultures. This explant was then placed into the center of each Petri dish. Controls without essential oils were prepared under the same conditions. Incubation was carried out at 26 °C. A measurement of each mycelial explant's growth diameter was done every 24 hours for 21 days. The means of 2 perpendicular diameters passing through the explants middle were calculated. These means were compared

to controls. Daily measurements were used to assess essential oils antifungal action on the mycelial growth of both *Phytophthora* strains (*P. palmivora* and *P. megakarya*). The inhibition rates of mycelial growth exerted by the essential oils were calculated according to the following formula (Whipps, 1997):

$$I. (\%) = (1 - D_n/D_o) \times 100$$

Where I. (%) is the mean inhibition rate; D<sub>n</sub> is the mean diameter of mycelial growth in the presence of essential oils and D<sub>o</sub> is the mean diameter of mycelial growth without essential oil (control).

#### **In vivo activity of essential oils**

##### ***Phytophthora* strains inoculum preparation**

The inoculum of *Phytophthora* strains was a zoospores suspension. This suspension was obtained from a 5 or 6-day-old culture carried out in Roux flasks. Incubation was carried out under a 12-hour photoperiod for 10 days. The cultures were flooded with 40 mL of distilled water and placed for 15 min at 4 °C. The cultures were then exposed for 45 min at room temperature to light from an incandescent lamp (60 W) in order to induce and accelerate sporogenesis (Babacauh, 1980). This heat shock operation allows the mature sporocysts to initiate the release of their zoospores. The zoospores suspensions obtained in each Roux flask were counted using a Malassez hemacytometer (SOVIREL, France) and calibrated to a concentration of 3×10<sup>5</sup> zoospores/mL for leaf discs inoculation (Mpika et al., 2009).

##### **Cocoa leaf discs test**

The leaf discs test was used to assess the effect of essential oils on *Phytophthora* strains as described by Nyassé et al. (1995). Healthy young leaves of clones (NA32, PA150 and SCA6) were collected from non-lignified twigs. These leaves were then preconditioned to make lamina more receptive to inoculation. The leaves were placed overnight underside down, against a foam soaked in distilled water, in a hermetically sealed tray. Leaf discs of 15 mm diameter were prepared and arranged in a four factors split-plot completely randomized design with four (04) repetitions for each treatment. Thus, four lines of ten (10) leaf discs or forty (40) leaf discs per clone, per essential

oil, per *Phytophthora* strain and per tray were inoculated (i.e.) 120 leaf discs for the three cocoa clones. For each treatment, leaf discs received 10 µL of each concentration of essential oils suspensions and 10 µL of *Phytophthora* zoospores suspensions. The controls received 20 µL of sterile distilled water. After inoculation, the trays were sealed with black plastic film and incubated at 26 °C in darkness for 7 days. Reduction rates (%) of leaf susceptibility were calculated using the following formula (Gadji et al., 2018):

$$R. (\%) = \frac{(A - B)}{A} \times 100$$

Where: R. (%) is the reduction rate of foliar susceptibility; A is the mean susceptibility indices of the control without essential oil; B is the mean foliar susceptibility indices of each treatment with the essential oils.

### Statistical analysis

The data obtained were analyzed with SAS version 9.4 software. The mean leaf susceptibility indices were compared using an analysis of variance. The Student Newman-Keuls test was used to make multiple comparisons at the 0.05 probability threshold.

## RESULTS

### *In vitro* effect of essential oils on the mycelial growth of two *Phytophthora* strains

Tables 1, 2 and 3 show the results of mycelial growth of both *Phytophthora* strains after addition of the essential oils to the culture media. From the analysis of these results, it appears that after 21 days of incubation, the essential oils showed antimicrobial activity against both *Phytophthora* strains. This activity varied according to *Phytophthora* strains, essential oils and concentrations. Indeed, the three essential oils exerted an important inhibitory activity towards *P. megakarya*. A total inhibition rate (100% inhibition) of mycelial growth was observed whatever the

concentration (0.5 to 5 mL/L). However, with *P. palmivora* strain, a differential activity was observed. Inhibition rates of 17.70% and 52.20% were observed with the essential oil of *Eucalyptus citriodora* species applied at concentrations of 0.5 and 1 mL/L. A total inhibition rate (100%) was noted with the concentration of 3 mL/L. Yet, with the essential oils of the species (*Cymbopogon citratus* and *Ocimum gratissimum*), high susceptibility of *P. palmivora* was observed at the minimum concentration of 0.5 mL/L and the inhibition rates ranged from 83.80 to 85.27%.

### *In vivo* effect of essential oils on cocoa clones susceptibility to *Phytophthora* strains

Tables 4, 5 and 6 show the results of leaf discs treatments with essential oils of the three aromatic plant species against *Phytophthora* strains. These results showed a significant difference ( $p < 0.05$ ) between leaf susceptibility indices induced by essential oils to the clones (NA32, PA150 and SCA6) and which of the controls. Indeed, essential oils induced a reduction of leaf susceptibility to *P. palmivora*. The mean susceptibility indices varied from 0.40 to 1.05 (no necrosis spots) compared to the index of 3.00 (presence of necrosis spots) of the control. Similarly, a reduction of leaf susceptibility to *P. megakarya* was observed. With *P. megakarya* strain, the mean susceptibility indices ranged from 0.42 to 1.11 compared to the index of 3.16 for control. These indices reflect reduction rates of foliar susceptibility to *Phytophthora* ranging from 64.87 to 86.70% with the three essential oils applied at doses of 0.5 to 5 mL/L (Table 7). However, these essential oils exerted more or less pronounced antifungal activity when applied at doses below 5 mL/L. Indeed, the essential oils (HO and HS) of *Ocimum gratissimum* and *Cymbopogon citratus* species exerted more pronounced antifungal activity than *Eucalyptus citriodora* species when applied at doses below 5 mL/L (Table 7).

**Table 1:** Inhibition rates of *Phytophthora* strains in the presence of essential oil from *Eucalyptus citriodora* species.

Essential oil concentrations (mL/L)	Inhibition rate (%) of <i>Phytophthora</i> strains mycelial growth	
	<i>Phytophthora palmivora</i>	<i>Phytophthora megakarya</i>
0,5	17,7	100
1	52,2	100
3	100	100
5	100	100

**Table 2:** Inhibition rates of *Phytophthora* strains in the presence of essential oil from *Cymbopogon citratus* species.

Essential oil concentrations (mL/L)	Inhibition rate (%) of <i>Phytophthora</i> strains mycelial growth	
	<i>Phytophthora palmivora</i>	<i>Phytophthora megakarya</i>
0,5	83,80	100
1	100	100
3	100	100
5	100	100

**Table 3:** Inhibition rates of *Phytophthora* strains in the presence of essential oil from *Ocimum gratissimum* species.

Essential oil concentrations (mL/L)	Inhibition rate (%) of <i>Phytophthora</i> strains mycelial growth	
	<i>Phytophthora palmivora</i>	<i>Phytophthora megakarya</i>
0,5	85,27	100
1	100	100
3	100	100
5	100	100

**Table 4:** Leaf susceptibility indices of clone NA32 after application of essential oils.

Clone		NA32							
<i>Phytophthora</i> strains		<i>Phytophthora palmivora</i>				<i>Phytophthora megakarya</i>			
Essential oil concentrations (mL/L)		HE	HO	HS	Control	HE	HO	HS	Control
0,5		2,03 <sup>b</sup>	1,03 <sup>de</sup>	0,60 <sup>ef</sup>	3,00 <sup>a</sup>	2,51 <sup>b</sup>	1,27 <sup>c</sup>	0,95 <sup>cd</sup>	3,16 <sup>a</sup>
1		1,50 <sup>c</sup>	0,85 <sup>def</sup>	0,70 <sup>def</sup>	3,00 <sup>a</sup>	2,23 <sup>b</sup>	1,18 <sup>c</sup>	0,87 <sup>cd</sup>	3,16 <sup>a</sup>
3		1,13 <sup>d</sup>	0,62 <sup>ef</sup>	0,47 <sup>f</sup>	3,00 <sup>a</sup>	1,28 <sup>c</sup>	0,91 <sup>cd</sup>	0,48 <sup>d</sup>	3,16 <sup>a</sup>
5		1,05 <sup>de</sup>	0,40 <sup>f</sup>	0,48 <sup>f</sup>	3,00 <sup>a</sup>	1,11 <sup>c</sup>	0,83 <sup>cd</sup>	0,42 <sup>d</sup>	3,16 <sup>a</sup>

Values with the same letter in the same column are not statistically different according to the Newman -Keuls test at the 5% probability level. HE = *Eucalyptus citriodora* essential oil; HO = *Ocimum gratissimum* essential oil; HS = *Cymbopogon citratus* essential oil; Control = treatment without oil.

**Table 5:** Leaf susceptibility indices of clone PA150 after application of essential oils.

Clone		PA150							
<i>Phytophthora</i> strains		<i>Phytophthora palmivora</i>				<i>Phytophthora megakarya</i>			
Essential oil concentrations (mL/L)		HE	HO	HS	Control	HE	HO	HS	Control
0,5		0,87 <sup>b</sup>	0,41 <sup>c</sup>	0,38 <sup>c</sup>	1,81 <sup>a</sup>	1,63 <sup>b</sup>	1,10 <sup>c</sup>	0,67 <sup>de</sup>	1,91 <sup>a</sup>
1		0,75 <sup>b</sup>	0,35 <sup>c</sup>	0,32 <sup>c</sup>	1,81 <sup>a</sup>	1,20 <sup>c</sup>	0,85 <sup>cde</sup>	0,56 <sup>e</sup>	1,91 <sup>a</sup>
3		0,42 <sup>c</sup>	0,28 <sup>c</sup>	0,17 <sup>c</sup>	1,81 <sup>a</sup>	0,93 <sup>cde</sup>	0,67 <sup>de</sup>	0,28 <sup>f</sup>	1,91 <sup>a</sup>
5		0,37 <sup>c</sup>	0,17 <sup>c</sup>	0,18 <sup>c</sup>	1,81 <sup>a</sup>	1,03 <sup>cd</sup>	0,63 <sup>e</sup>	0,17 <sup>f</sup>	1,91 <sup>a</sup>

Values with the same letter in the same column are not statistically different according to the Newman -Keuls test at the 5% probability level. HE = *Eucalyptus citriodora* essential oil; HO = *Ocimum gratissimum* essential oil; HS = *Cymbopogon citratus* essential oil; Control = treatment without oil.

**Table 6:** Leaf susceptibility indices of clone SCA6 after application of essential oils.

Clone		SCA6							
<i>Phytophthora</i> strains		<i>Phytophthora palmivora</i>				<i>Phytophthora megakarya</i>			
Essential oil concentrations (mL/L)		HE	HO	HS	Control	HE	HO	HS	Control
0,5		1,10 <sup>b</sup>	0,48 <sup>cd</sup>	0,28 <sup>e</sup>	1,57 <sup>a</sup>	1,85 <sup>a</sup>	1,17 <sup>b</sup>	0,65 <sup>cde</sup>	1,68 <sup>a</sup>
1		0,67 <sup>c</sup>	0,33 <sup>de</sup>	0,20 <sup>e</sup>	1,57 <sup>a</sup>	1,27 <sup>b</sup>	0,80 <sup>cd</sup>	0,51 <sup>def</sup>	1,68 <sup>a</sup>
3		0,55 <sup>c</sup>	0,13 <sup>e</sup>	0,16 <sup>e</sup>	1,57 <sup>a</sup>	1,00 <sup>bc</sup>	0,72 <sup>cde</sup>	0,31 <sup>ef</sup>	1,68 <sup>a</sup>
5		0,25 <sup>e</sup>	0,16 <sup>e</sup>	0,11 <sup>e</sup>	1,57 <sup>a</sup>	0,60 <sup>cde</sup>	0,45 <sup>def</sup>	0,15 <sup>f</sup>	1,68 <sup>a</sup>

Values with the same letter in the same column are not statistically different according to the Newman -Keuls test at the 5% probability level. HE = *Eucalyptus citriodora* essential oil; HO = *Ocimum gratissimum* essential oil; HS = *Cymbopogon citratus* essential oil; Control = treatment without oil.

**Table 7:** Reduction rates of leaf susceptibility of clone NA32 induced by essential oils.

Essential oils	<i>Phytophthora palmivora</i>		<i>Phytophthora megakarya</i>	
	Sensitivity rating	Reduction rate (%)	Sensitivity rating	Reduction rate (%)
<i>Phytophthora</i> (Controls without oils)	3,00	-	3,16	-
<i>Eucalyptus citriodora</i>	1,05	65a	1,11	64,87a
<i>Ocimum gratissimum</i>	0,40	86,66b	0,83	73,73b
<i>Cymbopogon citratus</i>	0,48	84b	0,42	86,70c

Values with the same letter in the same column are not statistically different according to the Newman -Keuls test at the 5% probability level.

## DISCUSSION

Essential oils significantly reduced mycelial growth *in vitro* and leaf susceptibility to both *Phytophthora* strains. This revealed the toxicity of essential oils towards *Phytophthora* strains. Indeed, the antifungal and insecticidal properties of essential oils from aromatic plant species (*Ocimum gratissimum*, *Eucalyptus citriodora* and *Cymbopogon citratus*) were reported by several research works (Camara et al., 2007; Camara, 2009; Camara et al., 2010; Degnon et al., 2013; Kassi et al., 2014; Muthukumar et al., 2016; Ouedraogo et al., 2016; Tia et al., 2019). Most essential oils have a broad spectrum of action against fungi, bacteria, insects, nematodes and viruses (Camara et al., 2010). Their activity could depend on the nature and chemical structure of their constituents (Camara et al., 2007). Thus, the presence of hydroxyl group in phenolic compounds would confer an antioxidant property to aromatic plants (Shahidi et al., 1992). Other studies have confirmed the inhibitory action of monoterpenes on the growth of microorganisms including *Phytophthora* (Camara et al., 2010; Soro et al., 2020). Monoterpenes would act by increasing microorganisms' cellular permeability (Arras and Usai, 2001) and by degrading their nucleic acids (Lambert et al., 2001). Antimicrobial activity of the essential oil from *Eucalyptus citriodora* species is due to various molecules

such as 1,8-cineole, citronellal, citronellol, citronellyl acetate, p-cymene, eucamalol, limonene, linalool,  $\alpha$ -pinene,  $\gamma$ -terpinene,  $\alpha$ -terpineol, alloocimene, and aromadendrene (Su et al., 2006; Batish et al., 2008; Liu et al., 2008; Traore et al., 2013). According to Camara et al. (2007), the different activities (fungistatic and fungitoxic) of the essential oil from *Ocimum gratissimum* species are mainly due to a chemical constituent present at 93.9% (eugenol). Eugenol is known to have antimicrobial activity (Camara et al., 2007). According to Kobenan et al. (2019), the chemical analysis of *Cymbopogon citratus* species essential oil showed a high proportion of oxygenated monoterpenes (73.1%) with a predominance of  $\alpha$ -citral (31.89%) and neral (39.33%).

*Phytophthora megakarya* strain was more sensitive *in vitro* to the effect of essential oils than *P. palmivora* strain. Indeed, the minimum concentration of total inhibition (CI<sub>100</sub>) of mycelial growth of *P. palmivora* strain was 1 mL/L for both essential oils from *Ocimum gratissimum* and *Cymbopogon citratus* species against a concentration of 3 mL/L for *Eucalyptus citriodora* species essential oil. However, the minimum concentration of these three essential oils that completely inhibited mycelial growth of the *P. megakarya* strain was 0.5 mL/L. This difference in susceptibility of the two

*Phytophthora* strains could be explained by the fact that the *Phytophthora megakarya* strain is slower growing *in vitro* compared to the *Phytophthora palmivora* strain (Mpika et al., 2009; Coulibaly et al., 2018; Ouattara et al., 2020). Thus, with the *Phytophthora megakarya* strain, the oils have time to act on the mycelial walls before the expiration of the strain's latency time, which varies from 48 to 72 hours at 26 °C in darkness. This is not the case for *Phytophthora palmivora* strain which has rather a short latency time (24 to 48 hours 26 °C in darkness); (Mpika et al., 2009; Coulibaly et al., 2018; Ouattara et al., 2020). Similar results were obtained by Soro et al. (2020), who observed *in vitro* a total inhibition of *Phytophthora palmivora* strain with a concentration (CI<sub>100</sub>) of 200 ppm with *Ocimum gratissimum* species essential oil. Doumbouya et al. (2012) observed 90% inhibition of mycelial growth of *Pythium* sp. with *Ocimum gratissimum* species essential oil at a concentration of 100 ppm (CI<sub>90</sub>).

### Conclusion

Essential oils from aromatic plant species (*Ocimum gratissimum*, *Eucalyptus citriodora* and *Cymbopogon citratus*) revealed reducing properties on mycelial growth of *P. palmivora* and *P. megakarya* strains. In addition, these oils also reduced *in vivo* the foliar susceptibility of clones (NA32, PA150 and SCA6) to both *Phytophthora* strains. Essential oils from *Cymbopogon citratus* and *Ocimum gratissimum* species were most effective than which from *Eucalyptus citriodora* species. The minimal dose of 0,5 mL/mL of essential oils from *Cymbopogon citratus* and *Ocimum gratissimum* species and the dose of 3 mL/mL for which of *Eucalyptus citriodora* species could be used for the control of black pod disease in field condition. These essential oils therefore revealed biofungicidal properties against *Phytophthora* and could be recommended as an alternative in the biological control of black pod disease in Côte d'Ivoire.

### COMPETING INTERESTS

The authors declare that they have no competing interests.

### AUTHORS' CONTRIBUTIONS

KC and FJMCK designed the protocol; AO and other authors participated in the study and contributed to the writing of the manuscript. DK and FKN supervised the study. All the authors read and approved the final version of the manuscript.

### ACKNOWLEDGEMENTS

We sincerely thank the Laboratory of Plant Physiology at Félix Houphouët-Boigny University for providing us with the essential oils. We also thank Mistere Coulibaly Bakary, Research technician, and Touré Laurent, Research assistant at the Laboratory of Plant pathology, National Center of Agronomic Research (CNRA), Research Station of Divo (Côte d'Ivoire), for the appreciable help provided during the leaf inoculation tests.

### REFERENCES

- Arras G, Usai M. 2001. Fungitoxic activity of 12 oils against four postharvest citrus pathogens: chemical analysis of *Thymus capitatus* oil and its effect in subatmospheric pressure conditions. *J. Food Prot.*, **64**(7): 1025-1029. DOI: <https://doi.org/10.4315/0362-028X-64.7.1025>
- Assiri AA, Kacou EA, Assi FA, Ekra S, Dji KF, Couloud JY, Yapo AR. 2012. Rentabilité économique des techniques de réhabilitation et de replantation des vieux vergers de cacaoyers (*Theobroma cacao* L.) en Côte d'Ivoire. *Journal of Animal and Plant Sciences*, **14**(2): 939-1951. <https://www.m.elewa.org/JAPS/2012/14.2/3.pdf>
- Babacauh KD. 1980. Structure et dynamique des populations de *Phytophthora* sp. Parasite du cacaoyer (*Theobroma cacao* L.). Thèse de Doctorat d'Etat, Université de Paris-Sud, centre d'Orsay, France, 153 p.
- Bailey BA, Ali SS, Meinhardt LW. 2016. *Phytophthora megakarya*, a Causal Agent

- of Black Pod Rot in Africa. In: *Cacao Diseases: A History of Old Enemies and New Encounters*, Bryan AB, Meinhardt LW (Eds). Springer International Publishing: Switzerland; 267-306. DOI:10.1007/978-3-319-24789-2
- Batish DR, Singh HP, Kohli RK, Kaur S. 2008. Eucalyptus essential oil as a natural pesticide. *Forest Ecology and Management*, **256**(12): 2166-2174. DOI: 10.1016/j.foreco.2008.08.008
- Camara A. 2009. Lutte Contre *Sitophilus Oryzae* L. (Coleoptera: Curculionidae) Et *Tribolium Castaneum* herbst (Coleoptera: Tenebrionidae) dans les stocks de riz par la technique d'étuvage traditionnelle pratiquée en Basse-Guinée et l'utilisation des huiles essentielles végétales. Thèse de Doctorat, en Sciences de l'Environnement, Université du Québec, Montréal, Canada, 173p.
- Camara B, Dick E, Sako A, Kone D, Kanko C, Boye M.-A.-D, Ake S, Anno A. 2010. Lutte biologique contre *Deightoniella torulosa* (Syd.) Ellis, par l'application des huiles essentielles d'*Eucalyptus platyphylla* F. Muell. et de *Melaleuca quinquenervia* L. *Phytothérapie et Ecologie*, **8**: 240-244. DOI: 10.1007/s10298-010-0568-3
- Camara B, Koné D, Kanko C, Anno A, Aké S. 2007. Activité antifongique des huiles essentielles de *Ocimum gratissimum* L., de *Monodora myristica* (Gaaertn) Dunal et de deux produits de synthèses (Impulse et Folicur), sur la croissance mycélienne et la production de spore *in vitro* de *Deightoniella torulosa* (Syd.) Ellis. *Revue Ivoirienne de Science et Technologie*, **09**: 190-198.
- Castro HG, Perini VBM, Santos GR, Lea TCAB. 2010. Evaluation of content and composition of the essential oil of *Cymbopogon nardus* (L.) in different harvest times. *Revista Ciência Agrônômica*. **41**(2): 308-314. DOI: <https://doi.org/10.1590/S1806-66902010000200020>
- CCC (Conseil Café-Cacao). 2014. Rapport de la session. Plateforme de partenariat public-privé de la filière café-cacao, troisième session plénière / Hôtel Du Golf, Abidjan. Côte d'Ivoire, 14p.
- Coulibaly K. 2014. Étude de la structure des populations de *Phytophthora* spp, agent de la pourriture brune des cabosses de cacaoyers (*Theobroma cacao* L.) en Côte d'Ivoire. Thèse de Doctorat en Pathologie et Physiologie Végétales. Université Félix Houphouët-Boigny. Abidjan, Côte d'Ivoire, 190p.
- Coulibaly K, Aka RA, Camara B, Kassin E, Kouakou K, Kébé BI, Koffi NK, Tahy MG, Walet NP, Guiraud SB, Assi ME, Kone B, N'Guessan KF, Koné D. 2018. Molecular Identification of *Phytophthora palmivora* in the Cocoa Tree Orchard of Côte d'Ivoire and Assessment of the Quantitative Component of Pathogenicity. *International Journal of Sciences*, **7**(8): 7-15. DOI: 10.18483/ijSci.1707
- Deberdt P, Mfegue CV, Tondje PR, Bon MC, Ducamp M, Hurard C, Begoude BAD, Ndoumbe-Nkeng M, Hebbar PK, Cilas C. 2008. Impact of environmental factors, chemical fungicide and biological control on cacao pod production dynamics and black pod disease (*Phytophthora megakarya*) in Cameroon. *Biological Control*, **44**: 149-159. DOI: <https://doi.org/10.1016/j.biocontrol.2007.10.026>
- Degnon RG; Faton A-N, Adjou ES, Tchobo FP, Dahouenon-Ahoussi E, Soumanou MM, Sohounhloue DCK. 2013. Efficacité comparée des huiles essentielles de deux plantes aromatiques dans la conservation post-fumage du Chinchard (*Trachurus trachurus*). *Journal of Animal & Plant Sciences*, **19**(1): 2831-2839.
- Doumbouya M, Abo K, Lepengue AN, Camara B, Kanko K, Aidara D, Kone D. 2012. Activités comparées *in vitro* de deux fongicides de synthèse et de deux huiles essentielles, sur des champignons telluriques des cultures maraîchères en Côte d'Ivoire. *Journal of Applied Biosciences*, **50**: 3520-3532. <http://m.elewa.org/JABS/2012/50/8.pdf>

- Friedel H.-A, Claudia H, Irene K, Pedro M, Mara M. 2016. Renforcer la compétitivité de la production de cacao et augmenter le revenu des producteurs de cacao en Afrique de l'Ouest et en Afrique central, 84p.
- Gadji AAG, Kouamé KG, Coulibaly K, Yapo OB, Aka AR, Brar KS, Tyagi R, Abo K. 2018. Effect of *Bacillus thuringiensis* var. *kurstaki* HD-1-based biopesticide on the pathogenicity of *Phytophthora palmivora*. *Journal of Biodiversity and Environmental Sciences*, **12**(5): 456-464.
- Gramlich A, Tandya S, Andres C, Chincheros PJ, Armengot L, Schneider M, Schulin R. 2017. Cadmium uptake by cocoa trees in agroforestry and monoculture systems under conventional and organic management. *Science of the Total Environment*, **580**: 677-686. DOI: <http://dx.doi.org/10.1016/j.scitotenv.2016.12.014>
- Huguenin G, Boccas B. 1971. Rôle de quelques facteurs dans la formation et la germination des zoospores chez le *Phytophthora palmivora* Bult. *Ann. Phytopathol.*, **3**(3) : 353-71.
- ICCO (International Cocoa & Chocolate Organisation). 2000. Célébration du cacao, 115 p.
- ICCO. 2019. Quarterly Bulletin of Cocoa Statistics, Vol. XLV, No.3, Cocoa year 2018/19. 1p.
- Jiménez-Reyesa MF, Carrascob H, Oleab AF, Silva-Moreno E. 2019. Natural compounds: a sustainable alternative to the phytopathogens control. *J. Chil. Chem. Soc.*, **64**(2): 4459- 4465. DOI: <http://dx.doi.org/10.4067/S07117-97072019000204459>
- Kareem OI. 2016. Cocoa exports' effects of the European Union's SPS measures. *International Journal of Sustainable Economy*, **8**(1): 76-89. DOI: 10.1504/IJSE.2016.073686
- Kassi MF, Badou JO, Tonzibo FZ, Salah Z, Bolou ABB, Camara B, Amari EGDN-L, Koné D. 2014. Potentiel antifongique de l'huile essentielle de *Ocimum gratissimum* dans la lutte biologique contre la maladie des raies noires du bananier causée par *Mycosphaerella fijiensis* Morelet (Mycosphaerellaceae). *Agronomie Africaine*, **26**(2) : 1-11. DOI: <https://www.ajol.info/index.php/aga/article/view/107197/97087>
- Kobenan KC, Kouakou BJ, Bini KKN, Kouakou M, Dick AE, Ochou OG. 2019. Effets des Huiles Essentielles de *Ocimum gratissimum* L. et de *Cymbopogon citratus* Stapf sur les Paramètres de Croissance et de Production du Cotonnier en Côte d'Ivoire. *European Journal of Scientific Research*, **154**(1): 21-35.
- Kouamé KG, Kouassi KN, Kassi FM, Bolou BBA, Tuo S, Kanko C, Kone D. 2015. Antifungal Activity of Essential Oils Extracted from *Monodora myristica* (Gaertn), *Ocimum gratissimum* L. and *Zingiber officinalis* Roscoe on Post-harvest Anthracnose of Mango Fruit (*Mangifera indica* L.) Variety Kent in Côte d'Ivoire. *International Journal of Sciences*, **4**(12): 8-18. DOI: 10.18483/ijSci.869
- Lambert RJW, Sjandamis PN, Coote PJ, Nychas GJE. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol.*, **91**: 453-62. DOI: 10.1046/j.1365-2672.2001.01428.x
- Liu X, Chen Q, Wang Z, Xie L, Xu Z. 2008. Allelopathic effects of essential oil from *Eucalyptus grandis* E. urophylla on pathogenic fungi and pest insects. *Front. Forestry China*, **3**: 232-236. DOI: <https://doi.org/10.1007/s11461-008-0036-5>
- Mpika J, Kébé IB, Issali AE, N'Guessan FK, Druzhinina S, Komon-Zélazowska M, Kubicek CP, Aké S. 2009. Antagonist potential of *Trichoderma* indigenous isolates for biological control of *Phytophthora palmivora* the causative agent of black pod disease on cocoa (*Theobroma cacao* L.) in Côte d'Ivoire. *African Journal of Biotechnology*, **8**(20): 5280-5293. DOI: 10.4314/ajb.v8i20.65962

- Muthukumar A, Sangeetha G, Naveenkumar R. 2016. Antimicrobial activity of essential oils against seed borne fungi of rice (*Oryza sativa* L.). *Journal of Environmental Biology*, **37**: 1429-1436.
- Nyassé S, Cilas C, Herail C, Blaha G. 1995. Leaf inoculation as an early screening test for cocoa (*Theobroma cacao* L.) resistance to *Phytophthora* black pod disease. *Crop Protection*, **14**(8): 657-663. DOI: [https://doi.org/10.1016/0261-2194\(95\)00054-2](https://doi.org/10.1016/0261-2194(95)00054-2)
- Ouattara A, Coulibaly K, Konate I, Kebe B.I, Beugre GAM, Tidou AS, Abdelkarim F.-M. 2020. Screening and Selection *in vitro* and *in vivo* of Cocoa Tree (*Theobroma Cacao* Linn) Endophytic Bacteria Having Antagonistic Effects against *Phytophthora* Spp. Fungal Agents Responsible of Black Pod Disease in Côte d'Ivoire. *Journal of Applied & Environmental Microbiology*, **8**(1): 25-31. DOI:10.12691/jaem-8-1-4
- Ouedraogo I, Sawadogo A, Nebie RC, Dakouo D. 2016. Evaluation de la toxicité des huiles essentielles de *Cymbopogon nardus* (L) et *Ocimum gratissimum* (L) contre *Sitophilus zeamais* Motsch et *Rhizopertha dominica* F, les principaux insectes nuisibles au maïs en stockage au Burkina Faso. *Int. J. Biol. Chem. Sci.*, **10**(2): 695-705. DOI: <http://dx.doi.org/10.4314/ijbcs.v10i2.2>
- Oussou KR. 2009. Étude chimique et activités biologiques des huiles essentielles de sept plantes aromatiques de la pharmacopée ivoirienne. Thèse de Doctorat Unique. Laboratoire de chimie organique et biologique, UFR SSMT, Université de Cocody-Abidjan. 241p.
- Oxenham SK, Svoboda KP, Walters DR. 2005. Antifungal activity of essential oil Basil (*Ocimum basilicum*). *Phytopathology*, **153**: 174-180. DOI: <https://doi.org/10.1111/j.1439-0434.2005.00952.x>
- Ploetz R. 2016. The Impact of Diseases on Cacao Production: A Global Overview. In *Cacao Diseases: A History of Old Enemies and New Encounters*, Bailey BA, Meinhardt LW (Eds). Springer International Publishing: Switzerland; Beltsville, USA: 33-59. DOI: 10.1007/978-3-319-24789-2
- Pohe J, Agneroh TA. 2013. L'huile des graines de neem, un fongicide alternatif à l'oxyde de cuivre dans la lutte contre la pourriture brune des cabosses de cacao en Côte d'Ivoire. *Journal of Applied Biosciences*, **62**: 4644-4652. DOI: 10.4314/jab.v62i0.86147
- Pohe J, Pohe SSW, Okou SFF. 2013. Association oxyde de cuivre et metalaxyl dans la lutte contre la pourriture brune des cabosses de cacao en Côte d'Ivoire. *Journal of Animal & Plant Sciences*, **16**(3): 2362-2368. DOI: <https://www.m.elewa.org/JAPS/2013/16.3/2.pdf>
- Santos GR, Brum RBCS, Castro HG, Gonçalves CG, Fidelis RR. 2013. Effect of essential oils of medicinal plants on leaf blotch in Tanzania grass. *Revista Ciência Agrônômica*, **44**(3): 587-593. DOI: <http://dx.doi.org/10.1590/S1806-66902013000300022>
- Shahidi F, Janitha PK, Wanasundara PD. 1992. Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.*, **32**: 67-103. DOI: <https://doi.org/10.1080/10408399209527581>
- Soro S, Ayolie K, Aye SEV, Fofana B, Silue N, Soro NA, Kone D. 2020. Six fungicidal formulations to control *Phytophthora palmivora* causal agent of brown rot of cocoa pods in Côte d'Ivoire. *Journal of Global Biosciences*, **9**(10): 8088-8101. <https://www.mutagens.co.in/jgb/vol.09/10/091010.pdf>
- Su YC, Ho CL, Wang IC, Chang ST. 2006. Antifungal activities and chemical compositions of essential oils from leaves of four eucalypts. *Taiwan J. For. Sci.*, **21**: 49-61.
- Ten Hoopen GM, Krauss U. 2016. Biological Control of Cacao Diseases In *Cacao Diseases: A History of Old Enemies and New Encounters*, Bailey BA, Meinhardt LW (Eds). Springer International

- Publishing: Switzerland; 511-566. DOI: 10.1007/978-3-319-24789-2
- Tia EV, Cisse M, Douan GB, Kone A. 2019. Etude comparée de l'effet insecticide des huiles essentielles de *Cymbopogon citratus* DC et d'*Ocimum canum* Sims sur *Cylas puncticollis* Boheman, un charançon de la patate douce. *Int. J. Biol. Chem. Sci.*, **13**(3): 1789-1799. DOI: 10.4314/ijbcs.v13i3.46
- Tiendrebeogo A, Ouedraogo I, Bonzi Skassankogno AI. 2017. Etude de l'activité antifongique d'extraits de *Cymbopogon citratus* (DC.) Stap, *Eclipta alba* L., *Lippia multiflora* M. et *Agave sisalana* P. *Int. J. Biol. Chem. Sci.*, **11**(3): 1202-1211. DOI: <https://dx.doi.org/10.4314/ijbcs.v11i3.22>
- Traore N, Sidibe L, Bouare S, Harama D, Somboro A, Fofana B, Diallo D, Figueredo G, J-C. Chalchat. 2013. Activités antimicrobiennes des huiles essentielles de *Eucalyptus citriodora* Hook et *Eucalyptus houseana* W.Fitzg. ex Maiden. *Int. J. Biol. Chem. Sci.*, **7**(2): 800-804. DOI: <http://dx.doi.org/10.4314/ijbcs.v7i2.34>
- Whipps JK. 1997. Development in the biological control of soil-borne plant pathogens. *Advances in Botanical Research*, **26**: 1-134. DOI: [https://doi.org/10.1016/S0065-2296\(08\)60119-6](https://doi.org/10.1016/S0065-2296(08)60119-6)
- Yapo KD, Ouffoue SK, N'guessan BR, Okpekon TA, Dade J, Say M, Kouako TH. 2014. Quality control by the determination of heavy metals in new variety of cocoa (cocoa mercedes) Côte d'Ivoire. *Journal de la Société Ouest-Africaine de Chimie*, **037**: 56-64.