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## **RESEARCH PAPER**

## **OPEN ACCESS**

Antifungal activity of the essential oil of *Melaleuca quinquenervia* (Cav.) ST against some pathogenic fungi of mango (*Mangifera indica* L.)

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## Abstract

In Senegal, mango is the most important fruit crop. It plays also a major role in exportation of the fruit and vegetables. Mango production faces however many phytosanitary problems. In fact, the mango tree is susceptible to a number of diseases at all stages of its development from planting to fruit. The control of mango diseases is essentially based on the use of chemical pesticides that have a negative impact on both the environment and human and animal health. In this context, a more popular trend consists with the use of environment friendly products as alternatives to chemical pesticides. In this study, the antifungal activity of the essential oil of leaves of *Melaleuca quiquenervia* on pathogenic fungi isolated from mango was explored and its composition analyzed. The essential oil showed a good antifungal activity with the highest mycelial inhibition rate  $(83.72\pm3.74\%)$  with *Lasiodiplodia theobromae* at 10000ppm. With *Fusarium* sp. and *Pestalotia* sp., the inhibition rates reached 74.70±3.67 and 69.45±26.48 respectively at 10000ppm. The major components of *Melaleuca* essential oil were 1.8-cineole (42.87%), viridiflorol (11.75%), limonene (10.11%),  $\alpha$ -pinene (8.74%),  $\alpha$ -terpineol (6.94%) and nerolidol (3.37%).

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### Introduction

In Senegal, mango accounts for 60% of fruit production. The area allocated to mango cultivation is estimated at more than 41 000 ha and the overall production of mangoes was 132 572 tons in 2013 (FAOSTAT, 2015). The mango sector is the most dynamic value chain in fruit export (Diouf. 2016). This sector employed nearly 30.000 people and generated an annual turnover of about 20 billion FCFA (ASEPEX, 2012). The mango-producing areas are mainly represented by the regions of Dakar, Thiès, Kaolack, Saint-Louis, Fatick, Kolda and Ziguinchor. In parallel with this production, mango exportations have evolved gradually since 1998. When from 300 tons, it increased up to 19.500 tons in 2019 (DPV, 2020). However, the Niayes area, which alone accounts for 80% of export volumes, is the main production site for mangoes for export. Phytosanitary constraints are today a major problem in the expression of the real potential of the mango sector in Senegal. Mango is susceptible to a number of diseases at all stages of its development from planting to fruit (Alemu, 2014), leading to a drop in yields and depreciation in the market value of mangoes. This depreciation of the quality of mango is mainly caused by pathogenic fungi. The main fungi responsible for post-harvest alterations are: Colletotrichum gloeosporioides, Alternaria alternata, Lasiodiplodia theobromae. Dothiorella sp, Aspergillus nige, Phoma sp. and Fusarium spp. (Dieve et al., 2021; Diedhiou et al., 2007; Mbaye, 2006). Among the fungal diseases responsible for fruit rot, anthracnose is the most important disease of mango.

Depending on the agro-climatic zones and the frequency of rainfall, the disease can cause heavy losses (Cissé *et al.*, 2020; Diedhiou *et al.*, 2014). Post-harvest rot affects more than 40% of mangoes produced during the rainy season in northern Senegal (Diedhiou *et al.*, 2007). The control of plant pathogenic fungi is based mainly on treatment with fungicides. However, the use of chemical pesticides may lead to health risks to humans as well as pollution of the environment in addition to adverse effects to non-target organisms and the development of resistant fungal strains (Leroux, 2003).

Therefore, developing and implementing alternative control strategies that are more respectful of the environment and the health of consumers becomes a necessity. Indeed, the current regulatory context strongly encourages the development of phytosanitary products of natural origin as an alternative to chemical control means (Deschepper, 2017). Natural products such as essential oils could be an interesting alternative to synthetic chemicals to control pathogenic fungi and at the same time minimize negative impacts on the environment and human health. Essential oils are known to contain a diversity of volatile molecules such as terpenes, terpenoids, aromatic and aliphatic compounds derived from phenol (Bakkali et al., 2008). Various studies have shown the antifungal activity of essential oils on a diversity of fungi (Nguefack et al., 2013; Zani et al., 1991; Amini et al., 2016; Goudjil et al., 2016; Ghalem. 2016; Singh and Pandey, 2018).

The aims of this study were to test the antifungal activity of *Melaleuca quiquienervia* essential oil on three pathogenic fungi of mango and to identify the compounds it is made of.

### Materials and methods

#### Fungal strains

The biological material consisted with isolates of *Fusarium* sp., *Lasiodiplodia theobromae* and *Pestalotia* sp. isolated from leaves, twigs and fruits of mango trees in the Casamance area of Senegal. *Fusarium* sp. was isolated from malformed flowers of mango trees from the Casamance zone where it represents a major phytosanitary problem.

#### Plant material

Fresh Leaves of *Melaleuca quiquenervia* were harvested at the *parc de Hann* in Dakar in 2021.

### Method of extracting essential oil

The essential oils were extracted by the steam training method. The extraction device is of the Clevenger type consisting of a pressure cooker containing water inside which the plant material is deposited in a perforated metal plate supported by an iron stool to avoid contact with water.

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Then the cooker is hermetically closed and the whole is brought to the boiling point. The aromatic molecules are released after the bursting of the plant cells by the water vapor stream produced by the boiling water. A water source from a tap connected to the refrigeration system allows the condensation of the vapor carrying the aromatic molecules. The mixture made of water and essential oil is the separated by passing through a decanting ampoule where they appear with the water at the bottom and the oil phase on top. The essential oil is then separated from the water and put in a tinted bottle to avoid possible denaturation by light.

### Determination of the yield of essential oil

The essential oil yield is determined based on the biomass of plant material used for extraction. The yields were determined according to the formula from Laghouiter *et al.* (2015). Rhe% = (Mhe/Mmv) \* 100

Rhe: Essential oil yield (%) Mhe: Mass of the essential oil obtained (in g) Mmv: Mass of plant material used (in g)

# Evaluation of inhibition potential of essential oil on mycelial growth of fungi

The amount of essential oil to be tested is put in an Erlenmeyer and mixed with tween 20 (0.1%). PDA is added to the medium so that the total volume of the solution is equal to 60ml for each concentration to be tested. The medium is poured into petri dishes under a laminar flow hood. After 24 hours. a 1.1cm diameter of agar disk colonized a seven-day-old strain culture of the fungal strain to be tested is transferred into a fresh petri dish containing PDA amended with the essential oil at the target concentration. The control boxes contain only tween and PDA. There were 3 replicates for each concentration and also the control treatment.

The assessment of mycelial growth was carried out every 24 hours by measuring the average of the perpendicular diameters passing through the middle of the petri dish. Based on these values, the percentage of inhibition of mycelial growth was calculated by using the formula developed by Djordjevic *et al.* (2013). GIR = [(GC-GT) /GC] ×100 GIR: Growth inhibition rate (%) GC: growth of the mycelium in control plates (in cm)

## Evaluation of the inhibition potential of essential oil on fungal spore germination

The solution to be tested is made of 30ml of sterile distilled water mixed with tween 20 (0.1%) and the essential oil at the desired ratio. This mixture is added to a petri dish of the culture of the fungus to be tested. After three minutes. The mycelium is scraped using a scalpel blade and then filtered twice through a double layer of gaze layed in a funnel to remove the mycelial fragments. A drop of the mixture was deposited between a slide and coverslip and the preparations were placed in a Petri dish containing moistened tissue to maintain a high relative humidity.

Observations were made every 24 hours for 2 days and three repetitions are performed for each concentration of essential oil which ranged from 100 to 1500ppm. The number of germinating spores was counted as based on the initiation of the germ tube.

### Determination of LC50 value of essential oils

The LC50 value is the dose of the ingredient that achieves a 50% inhibition of mycelial growth or spore germination. Inhibition rates are transformed into probit values using the probit transformation table. The LC50 values for each species are determined by performing a linear regression of the probit values as a function of the decimal logarithms of the concentrations used.

## Determination of the chemical composition of essential oils

Essential oil samples were analyzed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS). An Agilent-HP 6890 gas chromatograph was used with a flame ionization detector (FID). The column used is VF-WAX in molten capillary silica (60 m x 0.25mm x 0.5 $\mu$ m). The oven temperature was programmed at 60°C for 6 min with a flow rate of 2°C/min from 60°C to 250°C and maintained at 250°C for 20 min with split mode injection (1 $\mu$ l of a 10% solution in hexane). The temperature of the injector and detector was 280°C. Helium was the carrier gas (23 psis/SM – 30 psis/FID). The GC/MS analyses were performed under the same conditions as CPG-FID using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 selective mass detector. The separation of the different constituents was done using a VF-WAX capillary column ( $60m \ge 0.25mm \ge 0.5\mu m$ ). The oven temperature was programmed at  $60^{\circ}$ C for 6 min with a flow rate of  $2^{\circ}$ C/min from  $60^{\circ}$ C to  $250^{\circ}$ C and maintained at  $250^{\circ}$ C for 20 min with a split mode injection 1-10. The percentages are calculated from GIC/FID peaks areas without using correction factor.

### Statistical analysis

The data was analyzed with statical software R.3.2.3 software (R Core Team. 2015). A two-factor analysis of variance (ANOVA) was performed using the *aov* function of the agricolae package. The Student Newmann and Keuls Multiple Comparison test (SNK) was done using the SNK test function of the agricolae package.

### Results

Effects of Melaleuca quiquenervia essential oil on mycelial growth

*Melaleuca quinquenervia* essential oil shows good antifungal activity in all strains tested (table 1). The highest inhibition rate was noted with *Lasiodiplodia theobromae* ( $83.72\pm3.74\%$ ) at 10000ppm at the same concentration. Inhibition rates of 74.70±3.67 and 69.45±26.48 respectively were observed for *Fusarium* spp. and *Pestalotia* sp.

**Table 1.** Inhibition rate (%) of Melaleucaquinquenervia essential oil mycelial growth of Fusariumspp, Lasiodiplodia theobromae and Pestalotia sp.

Concentration (ppm)	Fusarium sp.	Lasiodiplodia theobromae	Pestalotia sp.
0	o±o d	0±0 e	0±0e
100	1.01±1.75 d	0±0e	0±0e
500	20.11±19.58 bcd	0±0e	5.49±5.56e
1000	15.4±7.07 cd	2.55±0.90e	20.78±16.65d
2000	23.30±2.54 bcd	9.21±5.30de	20.78±5.59cd
3000	24.55±3.72 bcd	17.25±4.936d	40.20±3.24c
4000	34.75±7.46 bc	38.23±7.69c	55.49±4.33b
6000	39.64±14.13 bc	62.94±14.31b	63.14±10.28ab
8000	47.67±4.50 b	75.49±8.57a	70±7.91ab
10000	69.45±26.48 a	83.72±3.73a	74.70±3.67a

For the same column means followed by the same letters are not significantly different according to the Newmann Keuls student test (n = 3. P < 0.05)

# *Effects of Melaleuca quiquenervia essential oil on spore germination*

The essential oil of *M. quinquenervia* inhibited spore germination in the three test fungi (Table 2). A total inhibition of spore germination of *Pestalotia* sp. was observed at 500ppm. Low germination rates of fungal spores of  $3.33\pm5.77\%$  and  $11.11\pm10.18\%$  were noted, respectively for *Fusarium* sp. and *Lasiodiplodia thebromae* at 1500ppm.

**Table 2.** Germination rate (%) of spores of *Fusarium*spp. Lasiodiplodia theobromae and Pestalotia sp. inpresence of the essential oil of Melaleuca quinquenervia.

Concentration (ppm)	<i>Fusarium</i> sp.	Lasidiplodia theobromae	Pestalotia sp.
0	81.91±8.95b	85.86±17.23a	42.98±12.53a
100	54.96±4.63	82.78±8.17a	40.87±24.57a
250	56.26±9.24c	42.94±15.66b	11.11±5.49b
500	46.74±12.22a	24.47±5.53b	6.82±1.69b
1000	7.5±6.61d	14.44±17.10b	10.87±10.44b
1500	3.33±5.77d	11.11±10.18b	o±ob

For the same column means followed by the same letters are not significantly different according to the Newmann Keuls student test (P < 0.05)

# LC50 values of the fungal species for spore gemination and mycelial growth

Table 3 shows the LC50s obtained in the different strains of pathogenic fungi responsible for diseases in mango trees.

For both for mycelial growth and spore germination. The lowest LC50 values were noted with *Pestalotia* sp. with 3890.45ppm and 323.59ppm respectively (table 3). *Lasiodiplodia theobromae* showed a lower LC50 value (5011.87ppm) than *Fusarium* sp. (7585.78ppm). The LC50 value was always lower for spore germination as compared to mycelial growth regardless of the fungal species. It could be noted that Fusarium sp. exhibited the higher LC50 value for mycelial growth. However it displayed the lowest LC50 (457.09ppm) for spore germination.

### Chemical composition of essential oil

The extraction yield of *Melaleuca quiquenervia* essential oil was 1.1%. Table 4 shows the chemical composition of *Melaleuca quinquenervia* essential oil. The main compounds found in *Melaleuca quiquenervia* essential oil are oxides (43.28%),

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monoterpenes (25.02%), cyclic sesquiterpenols (14.6%), terpene alcohols (8.50%), aliphatic sesquiterpenols (3.40%) and sequiterpenes (2.47%). The chemical analysis revealed one hundred and five compounds representing 99.94% of the total chemical composition and the major components were 1.8-Cineole (42.87%), viridiflorol (11.75%), limonene (10.11%),  $\alpha$ -pinene (8.74%),  $\alpha$ -terpineol (6.94%) and nerolidol (3.37%).

**Table 3.** LC50 value (ppm) of different fungalspecies for mycelial growth and spore germination.

	Fusarium sp.		Lasiodiplodia theobromae		Pestalotia sp.	
Huile	LC50	LC50	LCEO (MC)	LC50	LC50	LC50
Essentielle	(MC)	(SG)	LC50 (MC)	(SG)	(MC)	(SG)
MQ	7585.78	457.09	5011.87	562.34	3890.45	323.59
MC: Mycelia	l growth	SG: S	pore germina	tion		

**Table 4.** Chemical composition of *Melaleuca*quinquenervia essential oil.

N°	RT (min)	Compounds	%
1	3.9	Acetone	0.01
2	6.1	Methyl 2-Methylbutyrate	0.01
3	6.5	α-Pinene	8.74
4	6.6	α -Thuyene	0.08
5	7.4	α -Fenchene	0.02
6	7.6	Camphene	0.12
7	8.9	β-Pinene	2.8
8	9.4	Sabinene	0.01
9	11	β-Myrcene	0.95
10	11.1	α -Phellandrene	0.06
11	11.3	Psi-Limonene	0.04
12	11.8	α -Terpinene	0.34
13	12.7	Limonene	10.11
14	13.3	1.8-Cineole	42.87
15	14.4	Cis- β -Ocimene	0.01
16	15	Gamma-Terpinene	0.93
17	15.3	Trans- $\beta$ -Ocimene	0.39
18	16.3	P-Cymene	0.27
19	17	Terpinolene	0.42
20	20.2	6-Methyl-5-Hepten-2-One	0.01
21	21.1	Cis-oxyde de rose	0.01
22	21.9	Trans-oxide de rose	0.01
23	27.8	Menthone	0.02
24	29	Menthofurane	0.04
25	29.2	Ylangene	0.01
26	29.7	α -Copaene	0.03
27	31.5	Benzaldehyde	0.13
28	32	α -Gurjunene	0.07
29	33.3	Linalol	0.28
30	34	Neoisopulegol	0.07
31	24.1	Acetate de Menthyle	0.05
32	34.5	Isopulegol	0.21
<u>33</u>	35.3	Fenchol	0.07
34	35.9	Camphene Hydrate	0.03
35	36	β-Caryophyllene	1.07
36	36.5	Terpinene-4-Ol	0.67

2	0	2	9

N°	RT (min)	Compounds	%
37	36.7	Sesquiterpene	0.02
38	36.9	Aromadendrene	0.01
39	37.5	Methyl Benzoate	0.04
40	38	Trans-P-Menth-2-EN-1-OL	0.02
41	38.8	Menthol	0.08
42	38.9	Allo-Aromadendrene	0.23
43	39.5	Trans-Pinocarveol	0.02
44	39.9	Sesquiterpene	0.01
45	40.2	Zonarene	0.01
46	40.2	Ethyle Benzoate	0.04
47	40.4	α-HUMULENE	0.2
48	40.5	Citronellyl Acetate	0.01
<u>49</u>	40.7	Delta-TERPINEOL	0.14
50	40.8	Gamma-SELINENE	0.02
51	41.6	Neral	0.04
52	41.7	Sesquiterpene	0.05
53	42	Terpenyl Acetate	0.53
54	42.3	α -Terpineol	6.94
55	42.8	Sesquiterpene	0.02
56	43.1	Sesquiterpene	0.02
57	43.3	Germacrene D	0.15
58	43.7	Beta-Selinene	0.14
59	43.8	α -Muurolene	0.03
60	45.7	Delta-Cadinene	0.28
61	46.3	Citronellol	0.27
62	47	α -Amorphene	0.01
63	47.6	Sesquiterpene	0.02
64	47.9	Sesquiterpene Mw=202	0.01
65	49.9	Calamenene	0.01
66	50.9	Geraniol	0.02
67	51	P-Cymene-8-OL	0.01
68	53	Cis-P-Mentha-1.8-DIEN-2-OL	0.01
69	54.4	Sesquiterpenol	0.02
70	54.6	Methyl Thio Benzoate	0.2
71	55.1	Palustrol	0.13
72		Caryophyllene Oxide	
	57.9	~ ! !	0.32
73	57.9 59.3	Sesquiterpenol	0.32 0.03
<u>73</u> 74	57.9 59.3 59.6	Sesquiterpenol Epi-Globulol	0.32 0.03 0.03
73 74 75	57.9 59.3 59.6 60.4	Sesquiterpenol Epi-Globulol Ledol	0.32 0.03 0.03 0.78
73 74 75 76	57.9 59.3 59.6 60.4 60.8	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene	0.32 0.03 0.03 0.78 0.05
73 74 75 76 77	57.9 59.3 59.6 60.4 60.8 61.3	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol	0.32 0.03 0.03 0.78 0.05 3.37
73 74 75 76 77 78	57.9 59.3 59.6 60.4 60.8 61.3 61.8	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol	0.32           0.03           0.03           0.78           0.05           3.37           0.1
73 74 75 76 77 78 79	57.9 59.3 59.6 60.4 60.8 61.3 61.8 61.9	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol	0.32           0.03           0.78           0.05           3.37           0.1           0.03
73 74 75 76 77 78 79 80	57.9 59.3 59.6 60.4 60.8 61.3 61.8 61.9 62.3	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Cubenol	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.04
73 74 75 76 77 78 79 80 81 81	57.9 59.3 59.6 60.4 60.8 61.3 61.8 61.9 62.3 62.3	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Globulol	0.32 0.03 0.78 0.78 0.05 3.37 0.1 0.03 0.04 0.12
73           74           75           76           77           78           79           80           81           82	57.9 59.3 59.6 60.4 60.8 61.3 61.8 61.9 62.3 62.8 62.8 63.4	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Globulol Viridiflorol Cupial	0.32 0.03 0.78 0.78 0.05 3.37 0.1 0.03 0.04 0.12 11.75
73           74           75           76           77           78           79           80           81           82           83	57.9 59.3 59.6 60.4 60.8 61.3 61.8 61.9 62.3 62.8 63.4 63.4 63.4	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Globulol Viridiflorol Guaiol	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.04           0.12           11.75           0.47
73 74 75 76 77 78 79 80 81 81 82 83 83	57.9 59.3 59.6 60.4 60.8 61.3 61.8 61.9 62.3 62.8 63.4 63.6 63.4	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Globulol Viridiflorol Guaiol Sesquiterpenone	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.04           0.12           11.75           0.47
73           74           75           76           77           78           79           80           81           82           83           84           85	57.9 59.3 59.6 60.4 60.8 61.3 61.8 61.9 62.3 62.8 63.4 63.6 63.4 63.6 64.1 64.4	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Globulol Viridiflorol Guaiol Sesquiterpenone 10-Epi-Gamma-Eudesmol Pacificial	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.04           0.12           11.75           0.47           0.02           0.03
73           74           75           76           77           78           79           80           81           82           83           84           85           86	57.9 59.3 59.6 60.4 60.8 61.3 61.8 61.9 62.3 62.8 63.4 63.6 63.4 63.6 64.1 64.4 64.4	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Globulol Viridiflorol Guaiol Sesquiterpenone 10-Epi-Gamma-Eudesmol Rosifoliol	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.04           0.12           11.75           0.47           0.02           0.01
73           74           75           76           77           78           79           80           81           82           83           84           85           86           87	57.9 59.3 59.6 60.4 60.8 61.3 61.8 61.9 62.3 62.8 63.4 63.6 64.1 64.4 64.8 65.8	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Globulol Viridiflorol Guaiol Sesquiterpenone 10-Epi-Gamma-Eudesmol Rosifoliol Sesquiterpenol	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.04           0.12           11.75           0.47           0.02           0.01           0.05
73           74           75           76           77           78           79           80           81           82           83           84           85           86           87           82	57.9 59.3 59.6 60.4 60.8 61.3 61.8 61.9 62.3 62.3 62.3 62.8 63.4 63.4 63.6 64.1 64.4 64.8 65.8 66.8	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Globulol Viridiflorol Guaiol Sesquiterpenone 10-Epi-Gamma-Eudesmol Rosifoliol Sesquiterpenol Eudesmol Isomere	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.04           0.12           11.75           0.47           0.02           0.01           0.05           0.01           0.05
73           74           75           76           77           78           79           80           81           82           83           84           85           86           87           88           82	57.9 $59.3$ $59.6$ $60.4$ $60.8$ $61.3$ $61.8$ $61.9$ $62.3$ $62.3$ $63.4$ $63.6$ $64.1$ $64.4$ $64.8$ $65.8$ $66.9$ $66.9$ $67.2$	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Globulol Viridiflorol Guaiol Sesquiterpenone 10-Epi-Gamma-Eudesmol Rosifoliol Sesquiterpenol Eudesmol Isomere Epoxyde Sesquiterpenique	$\begin{array}{c} 0.32 \\ 0.03 \\ 0.03 \\ 0.78 \\ 0.05 \\ 3.37 \\ 0.1 \\ 0.03 \\ 0.04 \\ 0.12 \\ 11.75 \\ 0.47 \\ 0.02 \\ 0.01 \\ 0.05 \\ 0.01 \\ 0.02 \\ 0.01 \\ 0.02 \\ 0.$
73           74           75           76           77           78           79           80           81           82           83           84           85           86           87           89           90           91	57.9 $59.3$ $59.6$ $60.4$ $60.8$ $61.3$ $61.8$ $61.9$ $62.3$ $62.3$ $63.4$ $63.6$ $64.1$ $64.4$ $64.8$ $65.8$ $66.9$ $66.5$ $67.3$	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Cubenol Globulol Viridiflorol Guaiol Sesquiterpenone 10-Epi-Gamma-Eudesmol Rosifoliol Sesquiterpenol Eudesmol Isomere Epoxyde Sesquiterpenique Eugenol Camma Eudesmol	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.78           0.05           3.37           0.1           0.03           0.04           0.12           11.75           0.47           0.02           0.01           0.05           0.01           0.02           0.02           0.02           0.67
73         74         75         76         77         78         79         80         81         82         83         84         85         86         87         89         90         91         92	57.9 $59.3$ $59.6$ $60.4$ $60.8$ $61.3$ $61.8$ $61.9$ $62.3$ $62.8$ $63.4$ $63.6$ $64.1$ $64.4$ $64.8$ $65.8$ $66.9$ $66.5$ $67.3$ $67.3$	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Cubenol Globulol Viridiflorol Guaiol Sesquiterpenone 10-Epi-Gamma-Eudesmol Rosifoliol Sesquiterpenol Eudesmol Isomere Epoxyde Sesquiterpenique Eugenol Gamma-Eudesmol	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.78           0.05           3.37           0.1           0.03           0.04           0.12           11.75           0.47           0.02           0.01           0.05           0.01           0.02           0.02           0.02           0.67           0.67
73         74         75         76         77         78         79         80         81         82         83         84         85         86         87         88         90         91         92	$\begin{array}{c} 57.9\\ 59.3\\ 59.6\\ 60.4\\ 60.8\\ 61.3\\ 61.8\\ 61.9\\ 62.3\\ 62.8\\ 63.4\\ 63.6\\ 64.1\\ 64.4\\ 64.8\\ 65.8\\ 66.9\\ 66.5\\ 67.3\\ 67.4\\ 67.6\\ 67.4\\ 67.6\\ 67.2\\ \end{array}$	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Cubenol Globulol Viridiflorol Guaiol Sesquiterpenone 10-Epi-Gamma-Eudesmol Rosifoliol Sesquiterpenol Eudesmol Isomere Epoxyde Sesquiterpenique Eugenol Gamma-Eudesmol T-Cadinol	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.78           0.05           3.37           0.1           0.03           0.05           0.01           0.02           0.01           0.02           0.01           0.02           0.02           0.02           0.02           0.02           0.02           0.03
$\begin{array}{c} 73 \\ 74 \\ 75 \\ 76 \\ 77 \\ 78 \\ 79 \\ 80 \\ 81 \\ 82 \\ 83 \\ 84 \\ 85 \\ 86 \\ 87 \\ 88 \\ 89 \\ 90 \\ 91 \\ 92 \\ 93 \\ 94 \\ 93 \\ 94 \\ 94 \\ 94 \\ 94 \\ 94$	$\begin{array}{c} 57.9\\ 59.3\\ 59.6\\ 60.4\\ 60.8\\ 61.3\\ 61.8\\ 61.9\\ 62.3\\ 62.8\\ 63.4\\ 63.6\\ 64.1\\ 64.4\\ 64.8\\ 65.8\\ 66.9\\ 66.5\\ 67.3\\ 67.4\\ 67.6\\ 67.9\\ 67.6\\ 67.9\\ 67.6\\ 67.9\\ 67.6\\ 67.9\\ 68.9\\ 68.9\\ 68.9\\ 69.9\\ 68.9\\ 69.9\\$	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Globulol Viridiflorol Guaiol Sesquiterpenone 10-Epi-Gamma-Eudesmol Rosifoliol Sesquiterpenol Eudesmol Isomere Epoxyde Sesquiterpenique Eugenol Gamma-Eudesmol T-Cadinol Sesquiterpenol a Muurolol	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.78           0.05           3.37           0.1           0.03           0.05           0.01           0.02           0.01           0.02           0.01           0.02           0.01           0.02           0.67           0.07           0.16           0.06
$\begin{array}{c} 73 \\ 74 \\ 75 \\ 76 \\ 77 \\ 78 \\ 79 \\ 80 \\ 81 \\ 82 \\ 83 \\ 84 \\ 85 \\ 86 \\ 87 \\ 88 \\ 89 \\ 90 \\ 91 \\ 92 \\ 93 \\ 94 \\ 95 \\ \end{array}$	$\begin{array}{c} 57.9\\ 59.3\\ 59.6\\ 60.4\\ 60.8\\ 61.3\\ 61.8\\ 61.9\\ 62.3\\ 62.8\\ 63.4\\ 63.6\\ 64.1\\ 64.4\\ 64.8\\ 65.8\\ 66.9\\ 66.5\\ 67.3\\ 67.4\\ 67.6\\ 67.9\\$	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Globulol Viridiflorol Guaiol Sesquiterpenone 10-Epi-Gamma-Eudesmol Rosifoliol Sesquiterpenol Eudesmol Isomere Epoxyde Sesquiterpenique Eugenol Gamma-Eudesmol T-Cadinol Sesquiterpenol a -Muurolol Cadinol Isomere	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.78           0.05           3.37           0.1           0.03           0.05           0.01           0.02           0.01           0.02           0.01           0.02           0.01           0.02           0.07           0.16           0.06
$\begin{array}{c} 73 \\ 74 \\ 75 \\ 76 \\ 77 \\ 78 \\ 79 \\ 80 \\ 81 \\ 82 \\ 83 \\ 84 \\ 85 \\ 86 \\ 87 \\ 88 \\ 89 \\ 90 \\ 91 \\ 92 \\ 93 \\ 94 \\ 95 \\ 94 \\ 95 \\ 96 \\ 94 \\ 95 \\ 96 \\ 96 \\ 96 \\ 96 \\ 96 \\ 96 \\ 96$	$\begin{array}{c} 57.9\\ 59.3\\ 59.6\\ 60.4\\ 60.8\\ 61.3\\ 61.8\\ 61.9\\ 62.3\\ 62.8\\ 63.4\\ 63.6\\ 64.1\\ 64.4\\ 63.6\\ 64.1\\ 64.4\\ 64.8\\ 65.8\\ 66.9\\ 66.5\\ 67.3\\ 67.4\\ 67.6\\ 67.9\\ 68.3\\ 68.3\\ 68.3\\ 68.9\\ 68.3\\ 68.9\\ 68.3\\$	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Globulol Viridiflorol Globulol Viridiflorol Guaiol Sesquiterpenone 10-Epi-Gamma-Eudesmol Rosifoliol Sesquiterpenol Eudesmol Isomere Epoxyde Sesquiterpenique Eugenol Gamma-Eudesmol T-Cadinol Sesquiterpenol a -Muurolol Cadinol Isomere	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.78           0.05           3.37           0.1           0.03           0.05           0.01           0.02           0.01           0.02           0.01           0.02           0.07           0.16           0.08           0.02
$\begin{array}{c c} 73 \\ \hline 74 \\ \hline 75 \\ \hline 76 \\ \hline 77 \\ \hline 78 \\ \hline 79 \\ \hline 80 \\ \hline 81 \\ \hline 82 \\ \hline 83 \\ \hline 84 \\ \hline 85 \\ \hline 86 \\ \hline 87 \\ \hline 88 \\ \hline 86 \\ \hline 87 \\ \hline 88 \\ \hline 89 \\ \hline 90 \\ \hline 91 \\ \hline 92 \\ \hline 93 \\ \hline 94 \\ \hline 95 \\ \hline 95 \\ \hline 96 \\ \hline 97 \\ \hline 77 \\ \hline 78 \\ \hline 79 \\ \hline 80 \\ \hline 77 \\ \hline 78 \\ \hline 79 \\ \hline 80 \\ \hline 80 \\ \hline 81 \\ \hline 82 \\ \hline 80 \\ \hline 81 \\ \hline 80 \\ \hline 81 \\ \hline 80 \\ \hline 80 \\ \hline 90 \\ \hline 91 \\ \hline 92 \\ \hline 93 \\ \hline 94 \\ \hline 95 \\ \hline 95 \\ \hline 97 \\ \hline 97 \\ \hline 77 \\ \hline 78 \\ \hline 77 \\ \hline 77 \\ \hline 78 \\ \hline 77 \\ \hline 78 \\ \hline 77 \\ \hline 77 \\ \hline 77 \\ \hline 78 \\ \hline 77 \\ \hline 77 \\ \hline 77 \\ \hline 78 \\ \hline 77 \\ \hline 77 \\ \hline 77 \\ \hline 78 \\ \hline 77 \\ \hline 77 \\ \hline 77 \\ \hline 78 \\ \hline 77 \\ 77 \\ \hline 77 \\ \hline 77 \\ 77 \\ \hline 77 \\ 77$	$\begin{array}{c} 57.9\\ 59.3\\ 59.6\\ 60.4\\ 60.8\\ 61.3\\ 61.8\\ 61.9\\ 62.3\\ 62.8\\ 63.4\\ 63.6\\ 64.1\\ 64.4\\ 64.8\\ 65.8\\ 66.9\\ 66.5\\ 67.3\\ 67.4\\ 67.6\\ 67.9\\ 68.3\\ 68.9\\ 68.9\\ 69.5\\ 68.3\\ 68.9\\ 69.5\\ 60.5\\ 68.3\\ 68.9\\ 60.5\\ 68.3\\ 68.9\\ 60.5\\ 60.5\\ 68.3\\ 68.9\\ 60.5\\$	Sesquiterpenol Epi-Globulol Ledol Epoxy-6.7-Humulene Nerolidol Gleenol Épi-Cubenol Cubenol Globulol Viridiflorol Guaiol Sesquiterpenone 10-Epi-Gamma-Eudesmol Rosifoliol Sesquiterpenol Eudesmol Isomere Epoxyde Sesquiterpenique Eugenol Gamma-Eudesmol T-Cadinol Sesquiterpenol a -Muurolol Cadinol Isomere Sesquiterpenol a - Muurolol	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.78           0.05           3.37           0.1           0.03           0.05           0.01           0.02           0.01           0.02           0.07           0.16           0.08           0.02           0.12
$\begin{array}{c} 73 \\ 74 \\ 75 \\ 76 \\ 77 \\ 78 \\ 79 \\ 80 \\ 81 \\ 82 \\ 83 \\ 84 \\ 85 \\ 86 \\ 87 \\ 88 \\ 86 \\ 87 \\ 88 \\ 89 \\ 90 \\ 91 \\ 92 \\ 93 \\ 94 \\ 95 \\ 96 \\ 97 \\ 96 \\ 97 \\ 96 \\ 97 \\ 98 \\ 90 \\ 91 \\ 92 \\ 93 \\ 94 \\ 95 \\ 96 \\ 97 \\ 98 \\ 90 \\ 91 \\ 92 \\ 93 \\ 94 \\ 95 \\ 96 \\ 97 \\ 98 \\ 90 \\ 97 \\ 96 \\ 97 \\ 98 \\ 90 \\ 97 \\ 98 \\ 90 \\ 91 \\ 92 \\ 93 \\ 94 \\ 95 \\ 96 \\ 97 \\ 98 \\ 90 \\ 97 \\ 98 \\ 90 \\ 97 \\ 98 \\ 90 \\ 97 \\ 98 \\ 90 \\ 91 \\ 92 \\ 93 \\ 90 \\ 91 \\ 93 \\ 90 \\ 91 \\ 93 \\ 90 \\ 91 \\ 93 \\ 90 \\ 91 \\ 93 \\ 90 \\ 91 \\ 93 \\ 90 \\ 91 \\ 93 \\ 90 \\ 91 \\ 93 \\ 90 \\ 91 \\ 93 \\ 90 \\ 91 \\ 93 \\ 90 \\ 91 \\ 93 \\ 90 \\ 91 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 91 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 93 \\ 90 \\ 90$	$\begin{array}{c} 57.9\\ 59.3\\ 59.6\\ 60.4\\ 60.8\\ 61.3\\ 61.8\\ 61.9\\ 62.3\\ 62.8\\ 63.4\\ 63.6\\ 64.1\\ 64.4\\ 64.8\\ 65.8\\ 66.9\\ 66.5\\ 67.3\\ 67.4\\ 67.6\\ 67.9\\ 68.3\\ 68.9\\ 69.5\\$	Sesquiterpenol         Epi-Globulol         Ledol         Epoxy-6.7-Humulene         Nerolidol         Gleenol         Épi-Cubenol         Cubenol         Globulol         Viridiflorol         Guaiol         Sesquiterpenone         10-Epi-Gamma-Eudesmol         Rosifoliol         Sesquiterpenol         Eudesmol Isomere         Epoxyde Sesquiterpenique         Eugenol         Gamma-Eudesmol         T-Cadinol         Sesquiterpenol         a -Muurolol         Cadinol Isomere         Sesquiterpenol         a -Eudesmol         Residinol Isomere         Sesquiterpenol         a -Eudesmol	0.32           0.03           0.78           0.05           3.37           0.1           0.03           0.78           0.05           3.37           0.1           0.03           0.05           0.01           0.02           0.01           0.02           0.07           0.16           0.08           0.02           0.12

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N°	RT (min)	Compounds	%
99	70.5	α -Cadinol	0.06
100	71.4	Citronellic Acide	0.05
101	71.5	Eudesma-7-En-4-Ol	0.03
102	71.7	Eugenyl Acetate	0.18
103	73.4	Caryophylla-3.7-Dien-6-Ol	0.03
104	76.3	Farnesol	0.02
105	87.6	Phytol	0.02
		TOTAL	99.94

RT: Rétention time

### Discussion

Melaleuca quinquenervia essential oil had a marked activity against the three pathogenic fungi of mango (*Fusarium* spp., *Lasiodiplodia theobromae* and *Pestalotia* sp.). Its inhibition effect was detected on spore germination as well as on fungal mycelial. The antifungal activity varied among the pathogens tested.

Different studies showed antifungal activity of M. quinquenervia essential oil. Doumbouya et al. (2012) observed that the mycelial growth of Fusarium oxysporum was completely inhibited at 8500ppm in the presence of this essential oil. Cisse et al. (2020) obtained with this essential oil a total inhibition of the mycelial growth of Colletotrichum gloeosporioides at 10000ppm as well as a good inhibition of spore germination at 1500ppm. The antifungal activity of M. quinquenervia essential oil is related to its composition. The major components were 1.8-Cineole (42.87%), viridiflorol (11.75%), limonene (10.11%), alpha pinene (8.74%), alpha terpineol (6.94%) and nerolidol (3.37%). The chemical composition obtained is similar to that noted by Diallo et al (2020) on samples from the Mbao Forest and Lake Tanma. These secondary metabolites increase the cellular permeability of fungi by degrading their nucleic acids thus causing inhibition of mitochondrial energy metabolism (Arras and Usai. 2001; Lambert et al., 2001).

### Conclusion

The results of this study showed that *Melaleuca quinquenervia* essential oil had a good activity on three pathogenic fungi isolated from mango. The chemical analysis revealed one hundred and five compounds and 1.8-cineole, viridiflorol, limonene, alpha pinene, alpha terpineol and nerolidol was the most abondant constituents.

All these results show that *Melaleuca quinquenervia* essential oil could be effective biological alternative against pathogenic fungi of mango.

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