

## Chemical constituents and pesticide efficacy of two essential oil combinations of *Cymbopogon citratus* (DC.) Stapf and *Mentha piperita* L. from Western Burkina Faso

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**Abstract:** The resistance to synthetic pesticides in *Anopheles* mosquitoes and phytopathogenic fungi constitutes a major concern. The search for natural pesticides is further encouraged. Essential oils (EOs) of *Mentha piperita* and *Cymbopogon citratus* constitute a potential source of molecules with pesticidal activity but at high doses. Developing optimized combinations of these natural substances would allow their use as effective pesticides. This study aims to determine the chemical profile and pesticide efficacy of two types of essential oil combinations of *C. citratus* and *M. piperita*. CG/MS analyses show that the EO obtained by co-distillation of *C. citratus* and *M. piperita* (CC/MP: 80/20) mainly contains citral (49.26%),  $\beta$ -myrcene (10.98%), menthol (5.90%) and menthone (4.50%). The EO of *C. citratus* mainly contains citral (74.32%) and  $\beta$ -myrcene (13.66%). *M. piperita* EO mainly contains menthol (31.54%), menthone (20.27%), menthofuran (15.10%), and menthyl acetate (8.59%). In the mixture of the two pure EOs (CC/MP\*: 80/20), citral (59.45%),  $\beta$ -myrcene (10.97%), menthol (6.31%) and menthone (4.05%) were mainly identified. All the EOs inhibited 100% mycelial growth of *Macrophomina phaseolina* and *Phoma sorghina* at concentrations of 0.6 and 0.2%. At the concentration of 0.05%, the inhibition rates were 49.63, 39.52, 39.52, and 26.33% on *M. phaseolina*, 41.13, 40.90; 19.67 and 21.50% on *P. sorghina*, respectively, for the OEs of CC/MP, *C. citratus*, *M. piperita*, and CC/MP\*. All the EOs, except that of *M. piperita*, caused 100% mortality on the susceptible strain of *Anopheles gambiae* at a concentration of 1%. However, at the same concentration, mortality rates were 21.11, 2.47, 8.56, and 3.33% on the resistant strain, respectively, for EOs of CC/MP, *C. citratus*, *M. piperita*, and CC/MP\*. EO combinations contain a diversity of molecules that are obtained by co-distillation of plants and have the best pesticidal properties.

**Keywords:** *C. citratus*; *M. piperita*; Chemical composition; Essential oil combinations; Pesticide.

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## 1. Introduction

*Cymbopogon citratus* DC. Stapf and *Mentha piperita* L. are two aromatic plants of the Poaceae and Lamiaceae families. These two plants are in high demand by local populations for their flavoring properties and the management of various pathologies<sup>1-3</sup>. Previous studies have reported that the EO of *C. citratus* is mainly composed of citral with a content higher than 70%<sup>4-7</sup>. It also contains  $\beta$ -myrcene at a reasonably high level<sup>4-7</sup>. Biological tests have shown that this EO has insecticidal activity against several species of mosquitoes, including *A. gambiae*, a primary vector responsible for malaria in West Africa<sup>8,9</sup>. Somda et al. also showed that *C. citratus* EO would inhibit the mycelial growth of *P. sorghina* and *F. moniliforme* at a concentration of 8%<sup>10</sup>. The EO of *M. piperita* is mainly composed of menthol, menthone, isomenthone, menthofuran, and menthyl acetate but at varying levels depending on the area where the plant is collected<sup>5,11,12</sup>.

Degnon et al. reported that *M. piperita* EO from Benin was effective against the fungal flora of milk at concentrations 5 and 6.66  $\mu\text{L}/\text{mL}$ <sup>13</sup>. Benazzeddine also showed that *M. piperita* EO proved toxicity to *T. confusum* and *S. oryzae*<sup>14</sup>. Therefore, the EOs of *C. citratus* and *M. piperita* could be used as naturally occurring pesticides as alternatives to synthetic pesticides. Indeed, chemical control constitutes the main method of pathogen management. This method uses synthetic chemicals belonging to the pyrethroids, organochlorines, organophosphates, carbamates, benzimidazoles, aromatic hydrocarbons, and sterol biosynthesis inhibitors families<sup>15,16</sup>. However, long-term use and the uncontrolled use of these synthetic products promoted the development of pathogen resistance, food intoxication, and environmental pollution<sup>17,18</sup>.

Conversely, research has shown that plant essential oils can be used as environmentally friendly alternatives to synthetic insecticides<sup>19</sup> because of their biodegradability, insect selectivity, and low vertebrate toxicity<sup>20</sup>. Unfortunately, the large-scale use of EOs in general as a pesticide still faces certain constraints: low extraction yield, energy-intensive extraction, and the need to use large concentrations, which makes the cost very high<sup>21,22</sup>. The current challenge is to find optimized formulations that allow for using small portions of EO but are still sufficiently compelling<sup>23</sup>. For this purpose, we have shown in a recent study that the co-distillation of *C. citratus* and *M. piperita* made it possible to obtain an EO whose antioxidant properties are improved compared to those of pure EOs and the mixture of the two pure EOs<sup>24</sup>. The present work was therefore initiated with a view to determine the chemical profile and pesticide efficacy of two types of essential oil combinations of *C. citratus* and *M. piperita* on *Phoma sorghina* and *Macrophomina phaseolina*, two fungi responsible for the rotting of cereals in storage; as well as on *Anopheles gambiae*, the major vector responsible for

malaria in Burkina Faso and resistant to synthetic insecticides.

## 2. Methodology

### 2.1. Extraction of essential oils

The plant material subjected to the extraction of EOs consisted of aerial parts of *Mentha piperita* L. and *Cymbopogon citratus* (DC.) Stapf. Samples were collected in August 2019 at the Nazi-Boni University site (11°12' North; 4°24' West). They were identified under numbers N° 961 for *C. citratus* and 965 for *M. piperita*. They were deposited in the herbarium of Nazi-Boni University. Samples subjected to EOs extraction had been dried beforehand away from light for seven days and at room temperature.

The EOs were extracted by hydrodistillation using a Clevenger-type apparatus from the dry aerial parts of *C. citratus* and *M. piperita*, apart, then from their mixture (co-distillation or simultaneous distillation) in the mass proportions of 80% for *C. citratus* (CC) and 20% for *M. piperita* (MP) (CC/MP: 80/20). Thus, three EOs were extracted: pure EO of *C. citratus*, pure EO of *M. piperita*, and the mixture obtained by co-distillation (CC/MP). Several extractions were made per sample. The extraction yield was determined according to the following formula:  $R = \frac{m}{M} \times 100$ ; m is the mass of EO obtained, and M is the mass of vegetal material.

### 2.2. Analysis of the chemical profile of essential oils

The EOs were analyzed using gas chromatography and mass spectrometry (GC/MS). The Agilent 8860 brand chromatograph was used. It was coupled to an Agilent 5977B mass spectrometer equipped with a quadripolar analyzer. The separation of various constituents was carried out using a DBWAX capillary column of the polyethylene glycol type (60 m x 0.25 mm), the thickness of the film (0.25  $\mu\text{m}$ ) under the following experimental conditions: gas vector : (helium: 1  $\text{mL}\cdot\text{min}^{-1}$ ), ionization energy (70 eV), injector temperature (250°C), detector temperature (250°C). The oven is programmed from 60°C to 250°C for 10 minutes with a gradient of 2°C $\cdot\text{min}^{-1}$ . The injection is in split mode 1-10. The mass spectra obtained were compared with those of NIST database<sup>25</sup>. The Kovat index (KI) of the different constituents was calculated and compared with those in the literature<sup>26</sup>. In addition to the three EOs extracted, a mixture of pure EOs of *C. citratus* and *M. piperita* (CC/MP\*: 80/20) was made in the same proportions as that obtained by co-distillation and then analyzed.

### 2.3. Antifungal test of essential oils

The fungal strains subjected to antifungal testing consisted of *Phoma sorghina* and *Macrophomina phaseolina*. They were isolated from cowpea and corn, respectively, and bought in the markets of Bobo-Dioulasso (Burkina Faso). The isolation and identification of these fungi were carried out

according to the method described by Mathur and Kongsdal<sup>27</sup>.

The EOs and a synthetic fungicide (Caïman rouge P: Permethrin 25 g/kg + Thiram 250 g/kg) were tested according to the direct contact method described by Adjou and Soumanou<sup>28</sup>. Concretely, PDA culture medium was sterilized for 30 minutes at 120°C and then cooled to 45°C. Decreasing amounts of the EO were added to obtain concentrations of 0.6, 0.2, and 0.05%. The mixture was homogenized and then distributed 9 cm in diameter in Petri dishes at 25 mL per dish. After solidification of the culture medium, a mycelial cluster of 5 cm diameter of each fungal species was placed in the center of the Petri dish. PDA medium without EO was the negative control. The positive control consisted of PDA medium containing a synthetic fungicide and tested at the recommended concentration of 0.25%. Incubation conditions were: temperature (22 ± 2°C); 12 hours of UV light alternating with 12 hours of darkness. The inhibition percentages were determined on the 4<sup>th</sup> and 7<sup>th</sup> days after incubation, respectively, for *M. phaseolina* and *P. sorghina* using the formula  $I(\%) = \left(1 - \frac{D}{Dt}\right) \times 100$ ; D is the growth diameter of the mycelium in the culture medium containing EO and Dt, the growth diameter of the mycelium of the negative control<sup>29</sup>. The tests were carried out in quadruplets.

## 2.4. Insecticide test of essential oils

### 2.4.1. Mosquito strains

Mosquitoes were delivered by the insectarium of the Institute for Research in Health Sciences Western Regional Directorate (Bobo-Dioulasso, Burkina). Two strains of *Anopheles gambiae* mosquitoes were used to test the insecticidal efficacy of EOs :

- A susceptible reference strain (Kisumu) from Kenya ;

- A resistant strain was collected in the valley of Kou (11°24' N, 4°25' W) located about thirty kilometers north of the city of Bobo-Dioulasso. This strain is multi-resistant to DDT, dieldrin, and pyrethroids<sup>30</sup>.

The EOs were tested on impregnated Whatman paper according to the WHO Tube Insecticide Test Protocol described by Wangrawa<sup>31</sup>. Operating conditions were temperature (27 ± 2°C) and relative humidity (80 ± 10%). Deltamethrin 0.05%, a synthetic insecticide, was used as a positive control.

### 2.4.2. Impregnation of Whatman N°1 papers with essential oils

For each EO, concentrations of 0.01, 0.1, and 1% were prepared in acetone and then tested. 2 mL of each EO solution were poured homogeneously using a Pasteur pipette onto Whatman N°1 paper previously cut to the dimensions 15 cm × 12 cm to be adapted to the WHO tube. For each solution, 4 Whatman papers were impregnated. Whatman papers impregnated only with 2 mL of acetone constituted controls. Impregnated papers were dried in the laboratory for 10 minutes, then wrapped in aluminum foil and kept in the refrigerator at 4°C until use.

### 2.4.3. Test procedure

For each dose, 100 *Anopheles gambiae* females aged 3 to 5 days are exposed for 1 hour in test tubes (marked red) lined with papers impregnated with the EOs at the rate of 25 mosquitoes per tube. After this exposure period, the mosquitoes are transferred to observation tubes (marked green) lined with non-impregnated paper. Cotton soaked in 10% glucose is placed over each observation tube. A group of 25 mosquitoes exposed only to acetone constituted the negative control. The synthetic insecticide has been tested under the same conditions as the EOs. Mortality is determined after 24 hours of observation using Abbott's formula :

$$\text{Mortality rate} = \frac{\text{Mortality in EO} - \text{Mortality in control}}{100 - \text{Mortality in control}} \times 100$$

The tests were carried out in quadruplets.

## 2.5. Statistical analysis

Bioassay data were analyzed using the Microsoft Excel spreadsheet and reported as mean ± standard deviation. The ANOVA analysis of variance was performed using IBM SPSS 25.0 software. The comparison of the means was made at the 5% level by the Student-Newman-Keuls test (S-N-K test).

## 3. Results and discussion

### 3.1. Extraction yield of essential oils

The EO obtained by co-distillation was extracted with a 1.03 ± 0.03% yield. That of *M. piperita* EO is 1.29 ± 0.05%. This yield is much higher than that of the Benin species, which was 0.45%<sup>13</sup>. The OE of *C. citratus* has an extraction yield of 1.05 ± 0.08%. This yield is lower than that of the species from Central Burkina Faso (1.25%)<sup>5</sup>. Collection area, stage of plant development, season and/or plant environment,

and reactions over time within the plant would account for differences in the yield of the EOs from the same plant species<sup>13</sup>.

### 3.2. Chemical profile of essential oils

The chromatographic analyses of the EOs made it possible to identify the constituents in Table 1. The structures of the respective majority constituents of the EOs of *C. citratus* and *M. piperita* are presented in Figures 1 and 2. All EOs are mainly monoterpenic with a predominance of oxygenated monoterpenes.

The EO of *C. citratus* mainly contains geranial (41.49%), neral (32.83%), or 74.32% of citral and β-myrcene (13.66%). This chemical composition is comparable to that of *C. citratus* EO from Central Burkina Faso, the main constituents of which were geranial (48.1%), neral (34.6%), and myrcene (11.0%)<sup>5</sup>. Similarly, the EO of *C. citratus* from

Southern Benin mainly contained geranial (41.3%), neral (33%) and myrcene (10.4%)<sup>7</sup>.

The EO of *M. piperita* mainly contains menthol (31.54%), menthone (20.27%), menthofuran (15.10%), menthyl acetate (8.59%), and 1,8-cineole (3.8%). Bassole et al. also identified the same compounds in the EO of the species from Central

Burkina Faso<sup>2</sup>. The same compounds were also identified in the EO of *M. piperita* from Serbia but with a significant qualitative difference. Indeed, the EO from Serbia contained mainly menthol (37.4%), menthyl acetate (17.4%), menthone (12.7%), limonene (6.9%), menthofuran (6.8%) and 1,8-cineole (5.6%)<sup>32</sup>.

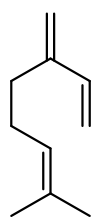
Geranial (26.90%), neral (22.36%),  $\beta$ -myrcene (10.98%), menthol (5.90%), and menthone (4.50%) were the major constituents of the EO obtained by co-distillation of the dry aerial parts of the two plants (CC/MP). This EO contains almost all of the major

compounds identified in the pure EOs of the two plants but at lower contents. However, some minor compounds contained only in the EO of *M. piperita*, such as  $\alpha$ -pinene (0.88%), 1,8-cineole (3.86%), and p-cymene (0.21%), have been identified with higher contents in the EO obtained by co-distillation (2.27; 4.26 and 3.30% respectively). Hay obtained comparable results on the EO from the mixture of the dry aerial parts of *R. officinalis*, *T. vulgaris*, and *C. officinalis*<sup>33</sup>. The mix of the two pure EOs (CC/MP\*) contains almost all the constituents identified in each pure EO but at lower contents. The low contents observed in the mixture of the pure EOs would be related to a dilution effect. However, in the EO obtained by co-distillation, the low contents observed of certain compounds would be linked, on the one hand, to a dilution effect and, on the other hand, to chemical reactions that would have occurred during the extraction process, which justifies the identification of different compounds at higher contents.

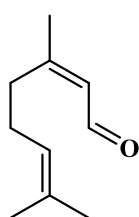
**Table 1.** Chemical composition of the essential oil obtained by co-distillation of *C. citratus* and *M. piperita* (CC/MP), the mixture of pure essential oils (CC/MP\*), and the essential oils of each plant.

KI	Compounds	Content (%)			
		<i>C. citratus</i>	<i>M. piperita</i>	CC/MP	CC/MP*
1025	$\alpha$ -pinene	-	0.88	<b>2.17</b>	0.18
1116	$\beta$ -pinene	-	1.06	0.24	0.21
1128	sabinene	-	0.53	-	0.11
1165	$\beta$ -myrcene	<b>13.66</b>	0.21	<b>10.98</b>	<b>10.97</b>
1170	$\alpha$ -phellandrene	-	-	<b>1.51</b>	-
1206	D-limonene	-	1.99	0.98	0.40
1214	$\beta$ -phellandrene	-	-	0.22	-
1220	1,8-cineole	-	<b>3.86</b>	<b>4.26</b>	0.77
1236	cis- $\beta$ -ocimene	0.36	-	0.26	0.29
1250	$\gamma$ -terpinene	-	0.21	0.32	-
1253	trans- $\beta$ -ocimene	0.25	-	-	0.20
1274	p-cymene	-	0.21	<b>3.30</b>	-
1340	6-methylhept-5-en-2-one	1.14	-	<b>1.74</b>	0.91
1398	3-octanol	-	0.46	-	-
1469	menthone	-	<b>20.27</b>	<b>4.50</b>	<b>4.05</b>
1481	lemonellal	0.17	-	-	0.14
1487	menthofuran	-	<b>15.1</b>	<b>2.87</b>	<b>3.02</b>
1497	isomenthone	-	<b>3.12</b>	0.58	0.62
1551	linalool	1.14	-	1.20	0.91

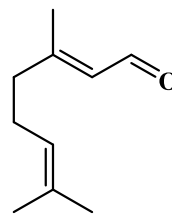
1568	menthyl acetate	-	8.59	1.55	1.72
1574	isoneral	1.46	-	1.11	1.17
1577	isopulegol	-	0.14	-	-
1602	2-undecanone	0.39	-	-	0.31
1607	terpinen-4-ol	-	0.57	0.51	0.11
1649	menthol	-	31.54	5.90	6.31
1651	pulegone	-	1.31	1.01	0.26
1668	(E)- $\beta$ -farnesene	-	0.19	-	-
1673	$\beta$ -humulene	-	0.20	-	-
1677	$\delta$ -terpineol	-	0.14	-	-
1688	neral	32.83	-	22.36	26.26
1702	$\alpha$ -terpineol	-	0.22	-	-
1712	germacrene D	-	0.71	-	0.14
1731	piperitone	-	0.30	0.38	-
1738	geranial	41.49	-	26.90	33.19
1739	(-)-carvone	-	0.59	-	0.12
1760	genanyl acetate	0.49	-	-	0.39
1771	lemonellol	0.31	-	0.22	0.25
1805	nerol	0.21	-	-	0.17
1812	2-tridecanone	0.23	-	0.24	0.18
1853	geraniol	3.49	-	2.05	2.79



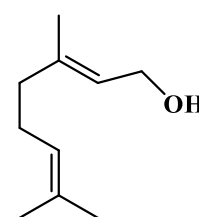
b-Myrcene (13,66%)



Neral (32,83%)

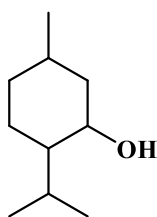
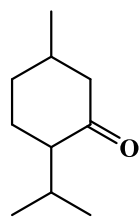
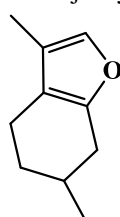
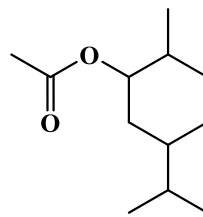
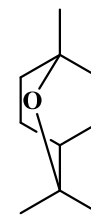


Geranial (41,49%)



Geraniol (3,49%)

a

Figure 1. Structure of the majority constituents of *C. ctratus* EOMenthol  
(31,54%)Menthone  
(20,27%)Menthofuran  
(15,10%)Menthyl acetate  
(8,59%)1,8-Cineole  
(3,86%)

b

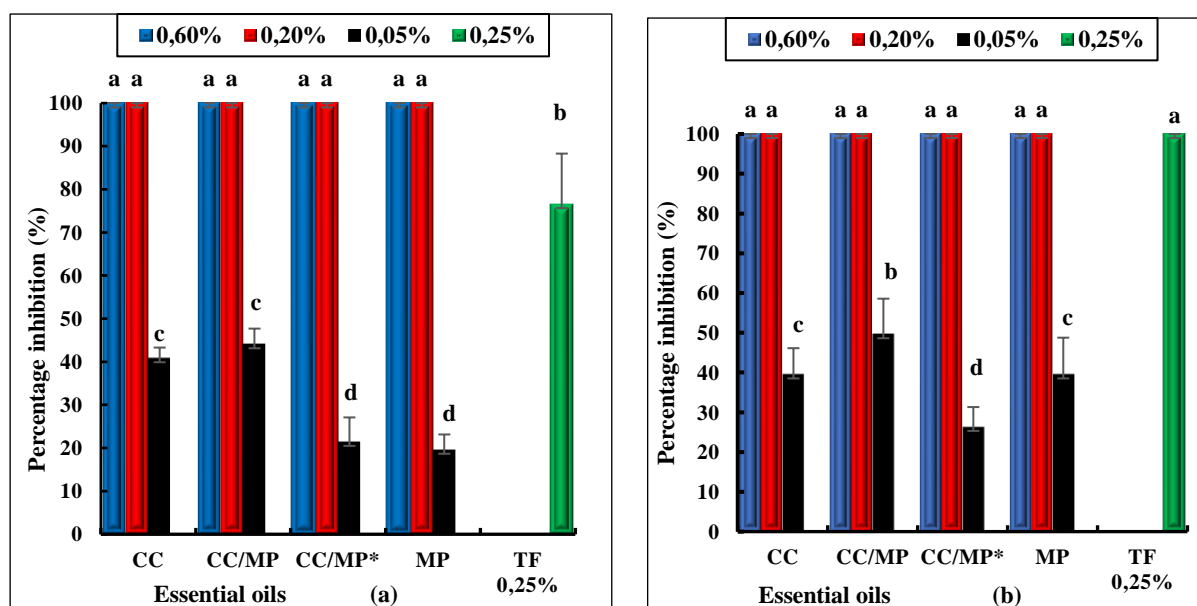
Figure 2. Structure of the majority constituents of *M. piperita* EO

### 3.3. Antifungal activity of essential oils

The results of the antifungal tests are shown in Figure 3. These results show that all EOs are effective against both fungi. ANOVA variance analysis indicates very significant differences between different treatments on *P. sorghina* and *M. phaseolina* ( $P=0.000$ ). At concentrations of 0.6 and 0.2%, all EOs inhibited 100% mycelial growth of both fungi. Synthetic fungicide has proved less effective than all EOs on *P. sorghina* (76.66% inhibition). Moreover, at the concentration of 0.05%, the EO obtained by co-distillation presented a higher inhibitory capacity on the mycelial growth of the two fungi than the pure EOs and the mixture of the pure EOs. However, the mix of the pure EOs was less effective than the pure EO of *C. citratus* on the two fungi.

The exciting antifungal efficacy of these EOs would be linked to their chemical composition, which is dominated by oxygenated monoterpenes (alcohols, ketones, and aldehydes), known for their antifungal solid activity. Many authors reported that monoterpene alcohols, aldehydes, and ketones are

active against microbial agents<sup>34,35</sup>. Indeed, EOs components are neurotoxic and affect acetylcholine,  $\gamma$ -aminobutyric acid, octopaminergic receptors, and respiratory pathways<sup>36</sup>. Moreover, the more marked antifungal activity of the EO obtained by co-distillation of plants compared to those of pure EOs and the mixture of pure EOs, could be linked to the different synergistic interactions of the diversity of molecules it contains. Indeed, p-cymene (0.21%), present only in the EO of *M. piperita*, was identified at a reasonably high content (3.30%) in the EO obtained by co-distillation. The latter could therefore act synergistically with citral (aldehyde), menthol (alcohol) and/or with menthone (ketone), thus increasing the antifungal efficacy of the EO. This analysis confirms that of Garia-Diez et al. who attributed the synergistic effect of combining pure EOs of thyme and cumin on *Salmonella* sp to the interaction between the p-cymene of cumin and thymol (alcohol) of thyme<sup>37</sup>. Similarly, Ultee et al. have reported a synergistic antimicrobial effect of the combination of p-cymene and carvacrol on *B. cereus*<sup>38</sup>.



**Figure 3.** Percentage inhibition of the EOs and synthetic fungicide (TF) on *P. sorghina* (a) and *M. phaseolina* (b) according to the doses

CC: EO of *C. citratus*; MP: EO of *M. piperita*; CC/MP: EO obtained by co-distillation; CC/MP\*: EO obtained by combining the pure EOs of the two plants; TF 0,25%: synthetic fungicide (Caiman rouge P) tested at 0.25%. According to the S-N-K test, bands assigned the same letter are not significantly different at the 5% threshold.

### 3.4. Insecticidal activity of essential oils

Figure 4 shows the percentages of mortality caused by the EOs and the synthetic insecticide (Deltamethrin 0,05%) on the sensitive (a) and resistant (b) strains of *An. gambiae*. The results show that all the EOs have insecticidal activity against the two strains of *An.*

*gambiae*. ANOVA variance analysis reveals significant differences between treatments on sensitive and resistant strains ( $P=0.000$ ). The EOs were more effective on the sensitive strain than the resistant strain. On the sensitive strain, all the EOs, except that of *M. piperita*, caused approximately 100% mortality at the dose of 1%. However, on the resistant strain, at the same dose, the EO of *C. citratus* and the mixture of the pure EOs, which had caused 100% mortality on the sensitive strain, only induced 2.47 and 3.30% mortality. The synthetic insecticide caused 100% mortality on the sensitive strain. However, on the resistant strain, the mortality rate was 14.84%. Similar results were obtained by Nonvhio et

al.<sup>8</sup>. Indeed, these authors reported that the EO of *C. citratus* from Benin had caused 100% mortality on the sensitive strain of *An. gambiae* at a dose of 22.6  $\mu\text{g}/\text{cm}^2$  had induced only 4% mortality on the resistant strain at the same dose. Insecticidal tests of the EO of *M. piperita* from Burkina Faso on adults of *An. gambiae* are a first. However, other studies have shown that EO from different origins is effective against insects such as *C. maculatus* and *C. chinensis*<sup>14,39</sup>.

The EO obtained by co-distillation proved the most effective on the resistant strain. Indeed, it was 8.5, 2.5, 6.3, and 1.4 times more effective than the EOs of *C. citratus*, and *M. piperita*, the mixture of the pure EOs and the synthetic insecticide. This result shows that the co-distillation made it possible to significantly improve the insecticidal efficacy of the EOs of these two plants. The difference in effectiveness from one strain to another for the same EO would be linked to

the difference in the genetic heritage of the two mosquito strains<sup>40</sup>. On the other hand, the complex chemical nature of the EOs could explain the difference in effectiveness between them for the same strain. Thus, the increased insecticidal efficacy of EO obtained by co-distillation compared to other EOs would be related to the molecular diversity it contains. Indeed, this EO contains molecules from the pure EO of co-distilled plants and molecules resulting from chemical transformations during co-distillation, particularly p-cymene, 1,8-cineole,  $\alpha$ -phellandrene, and  $\alpha$ -pinene. The insecticidal efficacy could, therefore, come from the synergistic actions of specific molecules, thus increasing the biological action of the EO. This analysis corroborates that of Wangrawa et al., who attributed the insecticidal efficacy of combinations of pure EOs on *A. gambiae* to new compounds previously absent in individual EOs<sup>40</sup>.

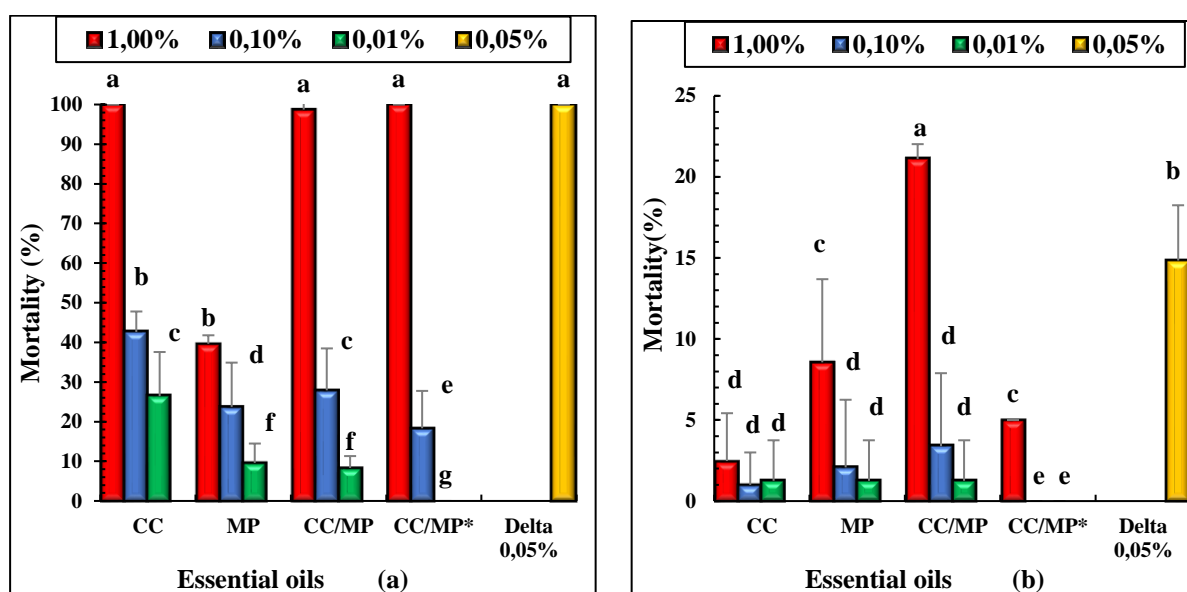


Figure 4. Mortality induced by the EOs on the sensitive strain (a) and the resistant strain (b)

CC: EO of *C. citratus*; MP: EO of *M. piperita*; CC/MP: EO obtained by co-distillation; CC/MP\*: EO obtained by combining pure EOs of the two plants; Delta0.05%: Deltamethrin 0.05%. According to the S-N-K test, bands assigned the same letter are not significantly different at the 5% threshold.

#### 4. Conclusion

This study shows that the pure EO of *C. citratus* contains mainly citral and  $\beta$ -myrcene. *M. piperita* mainly contains menthol, menthone, isomenthone, menthofuran, and menthyl acetate. The mixture of the two pure EOs contains almost all of the chemical constituents identified in each pure EO but in lower proportions. The EO obtained by co-distillation of the two plants also contains all the significant constituents identified in each pure EO but in lower contents. Yet, some minor compounds such as p-cymene, 1,8-cineole, and  $\alpha$ -pinene were identified with higher

contents. In vitro tests have shown that EO obtained by co-distillation has the best antifungal and insecticidal activities. The results of this study offer prospects for using this EO as a pesticide of natural origin that is effective and at a lower cost.

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