



COMPARATIVE STUDY OF THE INSECTICIDAL ACTIVITY OF ESSENTIAL OILS  
OBTAINED BY HYDRODISTILLATION OF DRIED LEAVES FROM TWO  
MYRTACEAE SPECIES CALLISTEMON VIMINALIS AND MELALEUCA  
LEUCADENDRA AGAINST SITOPHILUS ZEAMAI (MOTSCHULSKY, 1855)

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## ABSTRACT

**Background:** Post-harvest maize storage is threatened by *Sitophilus zeamais*, a major pest in sub-Saharan Africa, traditionally controlled using synthetic insecticides with recognized toxicological concerns. **Aim:** This study compared the bioinsecticidal potential of essential oils (EOs) from dried leaves of *Callistemon viminalis* and *Melaleuca leucadendra*, obtained by hydrodistillation and characterized by GC/MS. **Methods:** Insecticidal activity was evaluated against adult *S. zeamais* at four doses (D1-D4), with corrected mortality monitored over 29 days. Data were analysed using three-factor ANOVA (Plant × Dose × Time) followed by Tukey's test. **Results:** EO yield was significantly higher in *C. viminalis* ( $5.032 \pm 0.014\%$ ) than in *M. leucadendra* ( $1.684 \pm 0.210\%$ ). GC/MS revealed contrasting chemical profiles: *C. viminalis* contained phenylpropanoids (eugenol, meta-eugenol and methyleugenol; 16.94%) and phytol (12.21%), whereas *M. leucadendra* was dominated by hydrocarbon sesquiterpenes, mainly  $\beta$ -humulene and aromadendrene (53.01%), with no phenylpropanoids detected. *C. viminalis* produced rapid and pronounced insecticidal activity, reaching 99-100% mortality within 24 h, with an overall mean mortality of 81.00%. In contrast, *M. leucadendra* showed lower efficacy (40.7%) and a marked dose-dependent response. The difference was highly significant ( $F = 154.84$ ;  $p < 0.001$ ). **Conclusion:** *C. viminalis* appears more promising for botanical protection of stored maize, potentially due to the combined effects of its major bioactive constituents.

**KEYWORDS:** *Callistemon viminalis*, *Melaleuca leucadendra*, essential oil, *Sitophilus zeamais*, insecticidal activity, GC/MS.

## INTRODUCTION

The post-harvest storage of cereals is a major challenge for food security in sub-Saharan Africa, where losses due to insect pests can reach 20-30 per cent of stored production.<sup>[1, 2]</sup> Among these pests, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is recognised as the main pest of maize in storage<sup>[3, 4]</sup>, compromising

both the quantity and nutritional quality of the grains through its boring and egg-laying activities inside the grain.

Control of this pest has traditionally relied on the use of synthetic insecticides (notably organophosphates and pyrethroids), the prolonged use of which has led to the

development of resistance, risks of food contamination and negative impacts on the environment and the health of users.<sup>[5, 6]</sup> These constraints justify the search for effective, low-cost biological alternatives that are compatible with the farming practices of smallholder farmers.

In this context, essential oils from aromatic plants are attracting growing interest as alternative bioinsecticides. Their insecticidal activity, mainly attributed to terpenes and phenylpropanoids, acts via multi-target mechanisms on the nervous system of arthropods, notably by inhibiting octopaminergic receptors and acetylcholinesterase.<sup>[7, 8, 9]</sup> The Myrtaceae family, which is particularly rich in bioactive sesquiterpenes, oxygenated monoterpenes and phenylpropanoids, represents a phytochemical resource of interest in this regard.<sup>[10, 11]</sup>

### AIM

The present study focuses on the comparative evaluation of the insecticidal activity of essential oils from the dried leaves of two Myrtaceae species, *Callistemon viminalis* (Sol. ex Gaertn.) and *Melaleuca leucadendra* (L.), against adults of *Sitophilus zeamais*.

### OBJECTIVES

1. To determine the extraction yield and characterize the chemical composition of the essential oils from the dried leaves of *Callistemon viminalis* and *Melaleuca leucadendra* by hydrodistillation and GC/MS.
2. To evaluate the insecticidal activity of the essential oils against adult *Sitophilus zeamais* at different doses and exposure times.
3. To compare the insecticidal efficacy of the essential oils of *C. viminalis* and *M. leucadendra* using statistical analysis.
4. To assess the relationship between chemical composition and insecticidal activity and identify the most promising species for the post-harvest protection of stored maize.

### MATERIALS AND METHODS

#### Plant material

The plant material used in this study consists of leaves from two species of the Myrtaceae family: *Callistemon viminalis* (Sol. ex Gaertn.) and *Melaleuca leucadendra* (L.). The leaves of *C. viminalis* were collected from the botanical garden of the Faculty of Science and Technology at Cheikh Anta Diop University in Dakar (FST/UCAD), whilst those of *M. leucadendra* were collected from Hann Forest Park in Dakar. The leaves were harvested by hand from mature plants in good vegetative health, then washed with tap water and dried in the shade at room temperature for 7 days to remove residual moisture and stabilise the chemical composition of the plant material. Drying in the shade, which is preferred to oven drying, helps to limit the thermal degradation of heat-labile volatile compounds. The dried

leaves were then ground using an electric grinder prior to extraction.

#### Extraction of essential oils by hydrodistillation

The essential oils were extracted by hydrodistillation using a Clevenger-type apparatus, in accordance with the method described in the European Pharmacopoeia.<sup>[12]</sup> A specified mass of crushed dried leaves was placed in a 2-litre flask to which distilled water had been added. The apparatus was brought to the boil and maintained under reflux for 3 hours from the start of condensation of the first drop of oil. This duration was determined after verifying that the volume of oil collected remained constant, ensuring complete extraction of the volatile compounds. The essential oil, which is immiscible with water, separates naturally into the Clevenger's graduated tube. It was collected, dried over anhydrous sodium sulphate (Na<sub>2</sub>SO<sub>4</sub>) to remove any residual traces of water, then stored in hermetically sealed amber glass vials at 4 °C until use. The extraction rate (or essential oil yield) was calculated using the following formula.

$$\text{Extraction rate (\%)} = \frac{\text{mass of essential oil obtained}}{\text{mass of dry plant matter}} \times 100$$

#### Analysis by gas chromatography coupled with mass spectrometry (GC/MS)

The chemical composition of the essential oils was determined by gas chromatography coupled with mass spectrometry (GC/MS). The analyses were carried out on a gas chromatograph fitted with an HP-5MS non-polar capillary column (5% phenyl-polysiloxane, 30 m × 0.25 mm × 0.25 µm film thickness), coupled with an electron impact mass spectrometer (70 eV). The column temperature program ranged from 60° C to 280° C at a rate of 10° C/min. Helium was used as the carrier gas at a flow rate of 1 mL/min. Compounds were identified by comparing the mass spectra obtained with those in the NIST library. Only compounds with an identification quality factor ≥ 80 per cent were included.

#### Insecticidal bioassays on *Sitophilus zeamais*

The insecticidal activity of essential oils was assessed by contact bioassays on adults of *S. zeamais* Motschulsky, reared in mass culture under ambient conditions. Four doses (D1=100 µL, D2=75 µL, D3=50 µL, D4=25 µL) were tested on groups of 25 adult insects, with four replicates per treatment. Mortality rates were recorded at various time points (29 days) during exposure and corrected using Abbott's formula<sup>[13]</sup> to account for natural mortality. The data were subjected to a three-factor analysis of variance (ANOVA) (Plant × Dose × Time) using Minitab, followed by Tukey's multiple comparison test at the 5% significance level.

$$\text{Abbott's formula: } E = \frac{T - C_0}{100 - C_0} \times 100$$

- E: Adjusted treatment efficacy.
- T: percentage (or proportion) of organisms showing mortality observed in the treated batch.
- C<sub>0</sub>: percentage (or proportion) of mortality observed in the untreated control group.

## RESULTS

### Essential oil yield

Hydro-distillation of the dried leaves of the two Myrtaceae species yielded liquid essential oils that were slightly coloured (pale yellow to colourless) and had a characteristic odour. The extraction yields obtained are shown in Table 1.

**Table 1: Extraction yields of essential oils from dry leaves of *C. viminalis* and *M. leucadendra* by hydrodistillation (3 hours)**

| Species                      | Dry weight (g) | Oil content (mg) | Essential oil yield (%) |
|------------------------------|----------------|------------------|-------------------------|
| <b><i>C. viminalis</i></b>   | 200            | 10.06            | <b>5.032 ± 0.014</b>    |
| <b><i>M. leucadendra</i></b> | 400            | 6.74             | <b>1.684 ± 0.210</b>    |

The essential oil yield of *C. viminalis* is 5.032 ± 0.014%, which is approximately three times higher than that of *M. leucadendra* (1.684 ± 0.210%). These two figures demonstrate a marked difference in the abundance of volatile metabolites between the two species.

### Chemical composition of essential oils

GC/MS analysis of the essential oil from dried leaves of *C. viminalis* identified 17 compounds accounting for

88.82% of the total composition (Table 2). The dominant fraction is that of hydrocarbon sesquiterpenes (34.26%), followed by phenylpropanoids (16.94%), oxygenated sesquiterpenes (16.02%) and the diterpene phytol (12.21%). The most abundant compounds are phytol (12.21%), eugenol or meta-eugenol (10.10%),  $\alpha$ -terpineol (9.39%) and  $\beta$ -caryophyllene (9.28%).

**Table 2: Chemical composition of the essential oil from dried *C. viminalis* leaves (GC/MS)**

| Peak | RT (min) | Identified compound                     | %            | Chemical family           |
|------|----------|-----------------------------------------|--------------|---------------------------|
| 1    | 5.365    | <b><math>\alpha</math>-Terpineol</b>    | <b>9.39</b>  | Oxygenated monoterpene    |
| 2    | 6.694    | <b>Methyl eugenol / Eugenol</b>         | <b>10.10</b> | Phenylpropanoid           |
| 3    | 7.169    | <b>Methyleugenol</b>                    | <b>6.84</b>  | Phenylpropanoid           |
| 4    | 7.672    | <b><math>\beta</math>-Caryophyllene</b> | <b>9.28</b>  | Hydrocarbon sesquiterpene |
| 5    | 7.780    | Eremophilene                            | 4.20         | Hydrocarbon sesquiterpene |
| 6    | 7.914    | $\alpha$ -Caryophyllene (Humulene)      | 4.40         | Hydrocarbon sesquiterpene |
| 7    | 8.022    | Neoisolongifolene                       | 2.07         | Hydrocarbon sesquiterpene |
| 8    | 8.175    | $\alpha$ -Elemene                       | 1.85         | Hydrocarbon sesquiterpene |
| 9    | 8.390    | $\beta$ -Cadinene                       | 2.29         | Hydrocarbon sesquiterpene |
| 10   | 8.480    | $\delta$ -Cadinene                      | 2.34         | Hydrocarbon sesquiterpene |
| 11   | 8.776    | $\alpha$ -Calacorene                    | 3.28         | Hydrocarbon sesquiterpene |
| 12   | 8.938    | Epiglobulol                             | 2.53         | Oxygenated sesquiterpene  |
| 13   | 9.234    | (-)-Globulol                            | 3.52         | Oxygenated sesquiterpene  |
| 16   | 9.683    | $\tau$ -Cadinol                         | 3.48         | Oxygenated sesquiterpene  |
| 17   | 9.934    | Cadalene                                | 4.55         | Hydrocarbon sesquiterpene |
| 18   | 10.176   | <b>trans-Farnesol</b>                   | <b>6.49</b>  | Oxygenated sesquiterpene  |
| 19   | 13.219   | <b>Phytol</b>                           | <b>12.21</b> | Diterpene (alcohol)       |

As for the essential oil from the dry leaves of *M. leucadendra*, 12 compounds were identified, accounting for 82.86% of the total composition (Table 3). The dominant fraction is that of hydrocarbon sesquiterpenes (53.01%), followed by oxygenated sesquiterpenes (15.96%) and hydrocarbon monoterpenes (7.31%).  $\beta$ -Humulene is the most abundant compound (24.97%), followed by aromadendrene (17.59%),  $\alpha$ -eudesmol (15.96%),  $\delta$ -cadinene (7.14%) and  $\alpha$ -terpinolene (7.31%).

**Table 3: Chemical composition of the essential oil from dried leaves of *M. leucadendra* (GC/MS).**

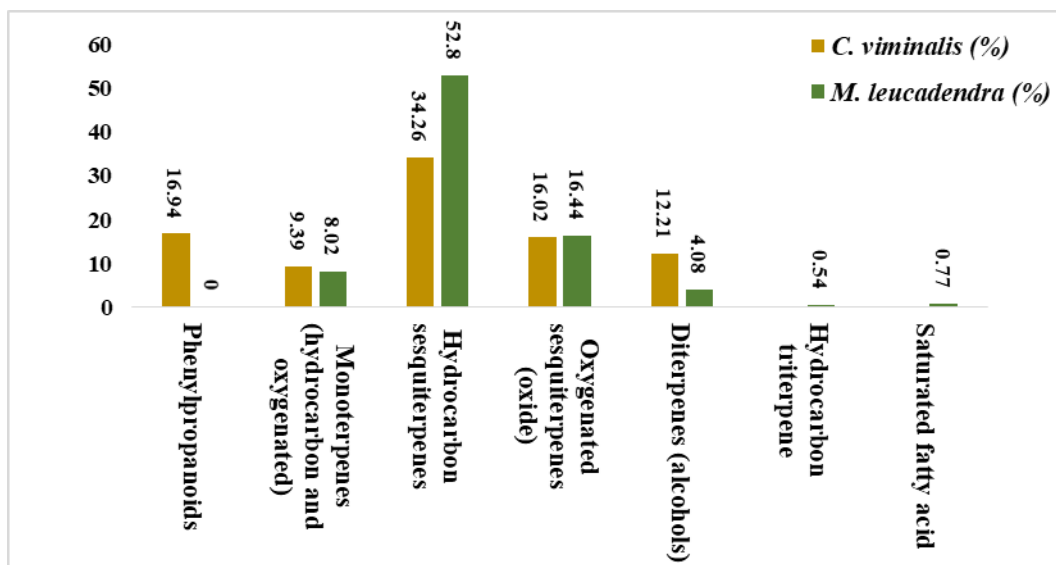
| Peak | RT (min) | Identified compound                    | %            | Chemical family                |
|------|----------|----------------------------------------|--------------|--------------------------------|
| 2    | 6.002    | Bornyl acetate                         | 0.71         | Oxygenated monoterpene (Ester) |
| 4    | 6.505    | <b><math>\alpha</math>-Terpinolene</b> | <b>7.31</b>  | Hydrocarbon monoterpene        |
| 5    | 7.232    | $\alpha$ -Gurjunene                    | 3.10         | Hydrocarbon sesquiterpene      |
| 6    | 7.403    | <b>Aromadendrene</b>                   | <b>17.59</b> | Hydrocarbon sesquiterpene      |
| 7    | 9.557    | <b><math>\beta</math>-Humulene</b>     | <b>24.97</b> | Hydrocarbon sesquiterpene      |
| 8    | 10.571   | <b><math>\delta</math>-Cadinene</b>    | <b>7.14</b>  | Hydrocarbon sesquiterpene      |
| 9    | 10.661   | <b><math>\alpha</math>-Eudesmol</b>    | <b>15.96</b> | Oxygenated sesquiterpene       |
| 13   | 11.388   | Isoaromadendrene epoxide               | 0.48         | Sesquiterpene oxide            |
| 14   | 12.008   | Hexadecanoic acid                      | 0.77         | Saturated fatty acid           |
| 15   | 13.175   | Phytol                                 | 3.60         | Diterpene (Alcohol)            |
| 16   | 13.803   | Geranylgeraniol                        | 0.48         | Diterpene (Alcohol)            |
| 20   | 18.408   | Squalene                               | 0.54         | Hydrocarbon triterpene         |

Figure 1 shows a comparative distribution of the chemical families identified in the two essential oils. The double-bar histogram allows the structural similarities and major phytochemical differences between the two extracts to be visualized simultaneously.

Both essential oils share the same dominant family, hydrocarbon sesquiterpenes, although their proportion is significantly higher in *M. leucadendra* (53.01%) than in *C. viminalis* (34.26%). Oxygenated sesquiterpenes also account for a comparable proportion in both extracts (16.02% versus 15.96%), although the constituent

compounds differ: globulol,  $\delta$ -cadinol and trans-farnesol in *C. viminalis*, versus  $\alpha$ -eudesmol in *M. leucadendra*.

The most striking chemical difference is the exclusive presence of phenylpropanoids in *C. viminalis* (16.94%), represented by eugenol, meta-eugenol and methyl eugenol. These compounds are completely absent from *M. leucadendra*. Conversely, hydrocarbon monoterpenes (7.31%), dominated by  $\alpha$ -terpinolene, are exclusive to *M. leucadendra* and absent from *C. viminalis*. The diterpene fraction is more abundant in *C. viminalis* (12.21%, mainly phytol) than in *M. leucadendra* (4.62%).

**Figure 1: Comparative analysis of the chemical families in the essential oils of dried leaves of *C. viminalis* and *M. leucadendra* (GC/MS).**

### Insecticidal activity

The insecticidal activity of two essential oils from the Myrtaceae family *Callistemon viminalis* (*C. vim.*) and *Melaleuca leucadendra* (*M. leu.*) was assessed by contact testing on *Sitophilus zeamais*, the main pest affecting maize stores in sub-Saharan Africa. The experimental design comprised three factors: plant (2 levels), dose (4 levels: D1 to D4) and exposure time (29 measurement

points). The response variable was the corrected mortality rate (Abbott). Statistical analyses were carried out using Minitab Statistical Software 22, employing a fixed-effects ANOVA and Tukey's multiple comparison test at the 5% significance level.

**Individual analysis: Two-factor ANOVA (Dose  $\times$  Time)**

### Callistemon viminalis: Maximum efficacy independent of dose

For *C. viminalis*, the ANOVA analysis shows that only the “Time” effect is significant ( $F = 7,690.36$ ;  $p < 0.001$ ). The “Dose” factors ( $p = 0.986$ ) and the “Dose  $\times$  Time” interaction ( $p = 1.000$ ) are completely insignificant. This result means that,

regardless of the dose applied, from the highest (D1) to the lowest (D4), mortality remains consistently high. The residual variance is very low (error standard deviation = 0.044), attesting to the robustness and consistency of the model. *C. viminalis* oil exerts a total lethal effect even at the lowest dose tested.

**Table 4: Individual ANOVA of *C. viminalis*, dry leaves**

| Factor             | DF  | Adj SS  | Adj MS  | F-Value | P-Value |
|--------------------|-----|---------|---------|---------|---------|
| Dose               | 3   | 0.01    | 0.002   | 0.05    | 0.986   |
| Time               | 28  | 9502.33 | 339.369 | 7690.36 | 0.000   |
| Dose $\times$ Time | 84  | 0.18    | 0.002   | 0.05    | 1.000   |
| Error              | 348 | 15.36   | 0.044   |         |         |
| Total              | 463 | 9517.87 |         |         |         |

Tukey’s test shows that doses D1 to D4 form a single homogeneous group (A), with a constant mortality rate of between 80 and 81%, indicating complete saturation

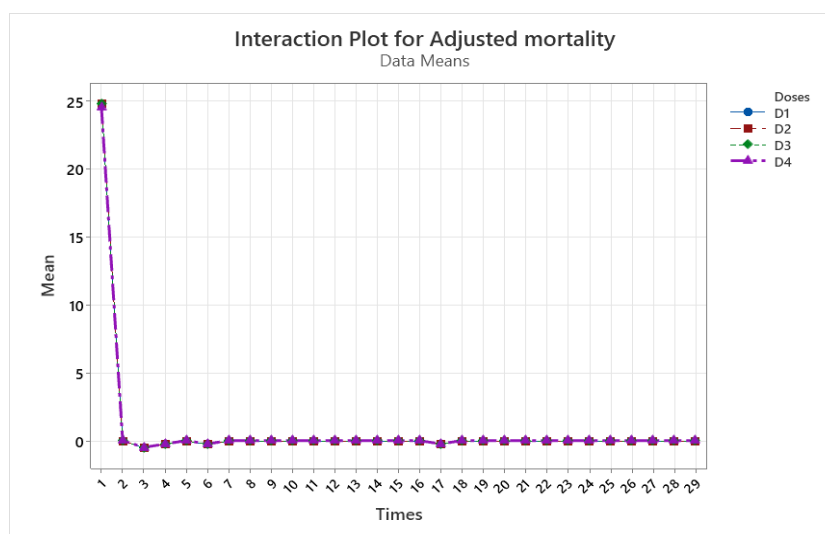
of the lethal effect from the minimum dose onwards. The oil is therefore active at very low concentrations.

**Table 5: Tukey’s comparisons of doses, *C. viminalis* (95% CI)**

| Doses | N   | Mean   | Grouping |
|-------|-----|--------|----------|
| D1    | 116 | 0.8120 | A        |
| D2    | 116 | 0.8120 | A        |
| D3    | 116 | 0.8120 | A        |
| D4    | 116 | 0.8034 | A        |

An analysis of mortality in *Sitophilus zeamais* exposed to the hydrodistilled essential oil of dried *Callistemon viminalis* leaves reveals a rapid insecticidal effect, characterized by total and immediate mortality of the insects within the first 24 hours of exposure, regardless of the dose tested. Indeed, the corrected cumulative mortality rate reached 99-100% by the first day for all four doses (D1 to D4), and remained at this level until the end of the trial (29 days). This result is reflected graphically by the mortality curves for the four doses

overlapping on the “Dose  $\times$  Time” interaction plot (Figure 2), which drops sharply between time 1 and time 2 before stabilizing around zero for the remainder of the observation period. The occasionally negative values observed at certain time points (day 3, day 4, day 6, day 17) do not reflect a real biological effect, but result from the application of Abbott’s correction formula when the control mortality, although low, exceeds a treated mortality that is already zero due to the depletion of the susceptible population.



**Figure 2: Dose  $\times$  time interaction plot for *Callistemon viminalis*.**

### Melaleuca leucadendra: Increasing dose-response relationship and significant interaction

For *M. leucadendra*, the three sources of variation are highly significant: “Dose” ( $F = 30.05$ ;  $p < 0.001$ ), “Time” ( $F = 205.42$ ;  $p < 0.001$ ) and the “Dose  $\times$  Time”

interaction ( $F = 49.88$ ;  $p < 0.001$ ). The significant interaction indicates that the effect of dose changes over the course of exposure. The residual variance (CM error = 0.443) is ten times greater than that of *C. viminalis*, reflecting greater variability in the biological response.

**Table 6: Individual ANOVA of *M. leucadendra*, dry leaves.**

| Factor      | DF  | Adj SS  | Adj MS | F-Value | P-Value |
|-------------|-----|---------|--------|---------|---------|
| Doses       | 3   | 39.90   | 13.301 | 30.05   | 0.000   |
| Times       | 28  | 2545.66 | 90.917 | 205.42  | 0.000   |
| Doses*Times | 84  | 1854.31 | 22.075 | 49.88   | 0.000   |
| Error       | 348 | 154.02  | 0.443  |         |         |
| Total       | 463 | 4593.89 |        |         |         |

Tukey’s test clearly distinguishes between two groups: D1 and D2 (high doses, group A: 69-70% mortality) versus D3 and D4 (low doses, group B: 7-16%). This dose-response relationship is increasing and biologically

consistent: high concentrations of essential oil cause high mortality, whilst low concentrations are insufficient to induce significant lethality in *S. zeamais*.

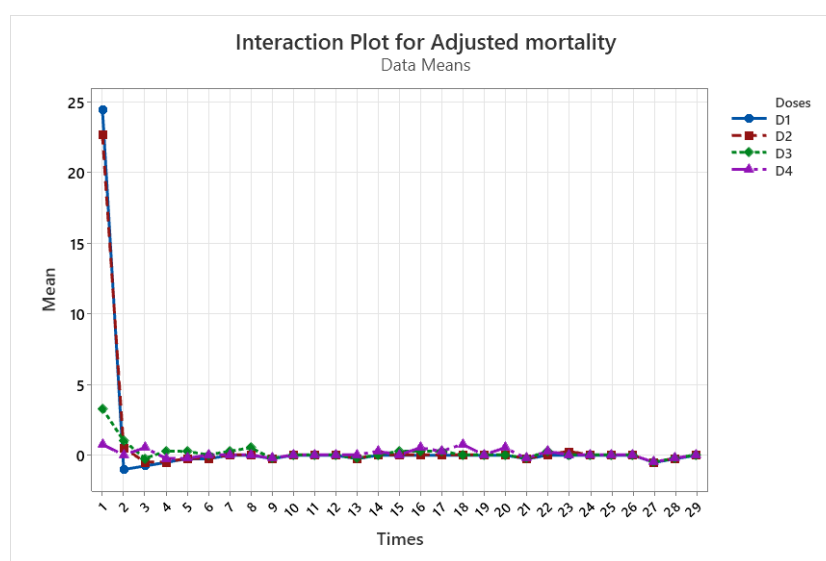
**Table 7: Tukey’s comparisons of doses, *Melaleuca leucadendra* (95% CI).**

| Doses | N   | Mean   | Grouping |
|-------|-----|--------|----------|
| D1    | 116 | 0.6937 | A        |
| D2    | 116 | 0.7025 | A        |
| D3    | 116 | 0.1639 | B        |
| D4    | 116 | 0.0673 | B        |

The “Dose  $\times$  Time” interaction plot generated by Minitab shows interval-adjusted mortality (the number of new deaths between two observations), which explains why the four doses appear to produce a visually similar effect at time 1 (peaks ranging from 22 to 25) before converging towards zero from the 1<sup>st</sup> and 2<sup>nd</sup> days onwards: this low plateau does not indicate comparable efficacy between the doses, but simply reflects the fact that no further deaths occur after the initial peak, regardless of the dose. Analysis of the raw Excel data, converted to adjusted cumulative mortality (using Abbott’s formula), actually reveals a clear dose-

dependent hierarchy masked by the interval plot: D1 and D2 reach 100% cumulative mortality by the 1<sup>st</sup> and 3<sup>rd</sup> days respectively, whilst D3 and D4 plateau at only 23.8% and 10.0% by the 29<sup>th</sup> day, with the majority of insects exposed to these lower doses therefore remaining alive throughout the observation period.

This contrast highlights a clear dose-response relationship ( $D1 > D2 \gg D3 > D4$ ) that can only be revealed by analysing cumulative mortality, as the interval interaction plot gives a misleading impression of homogeneity between the doses.



**Figure 3: Dose  $\times$  time interaction plot for *Melaleuca leucadendra*.**

### Overall analysis: Three-factor ANOVA (Plant × Dose × Time)

The three-factor analysis of variance (Table 8) covering the entire dataset (N = 928 observations) reveals that all main effects and interactions are highly significant ( $p < 0.001$ ). The “Time” effect is by far the most significant ( $F = 1604.21$ ), reflecting a progressive accumulation of

mortality over the course of exposure. The “Plant” effect ( $F = 154.84$ ) confirms a substantial difference in efficacy between the two extracts. The three-way interaction “Plant × Dose × Time” ( $F = 44.79$ ) indicates that the dose-response profiles of the two essential oils diverge over time, justifying their individual analysis.

**Table 8: Three-factor ANOVA: Hydrodistillation of dried leaves (Myrtaceae).**

| Source              | DF  | Adj SS  | Adj MS  | F-Value | P-Value |
|---------------------|-----|---------|---------|---------|---------|
| Plant               | 1   | 37.7    | 37.681  | 154.84  | 0.000   |
| Dose                | 3   | 20.3    | 6.765   | 27.80   | 0.000   |
| Time                | 28  | 10931.0 | 390.393 | 1604.21 | 0.000   |
| Plant × Dose        | 3   | 19.6    | 6.538   | 26.87   | 0.000   |
| Plant × Time        | 28  | 1117.0  | 39.892  | 163.92  | 0.000   |
| Dose × Time         | 84  | 938.9   | 11.178  | 45.93   | 0.000   |
| Plant × Dose × Time | 84  | 915.5   | 10.899  | 44.79   | 0.000   |
| Error               | 696 | 169.4   | 0.243   |         |         |
| Total               | 927 | 14149.4 |         |         |         |

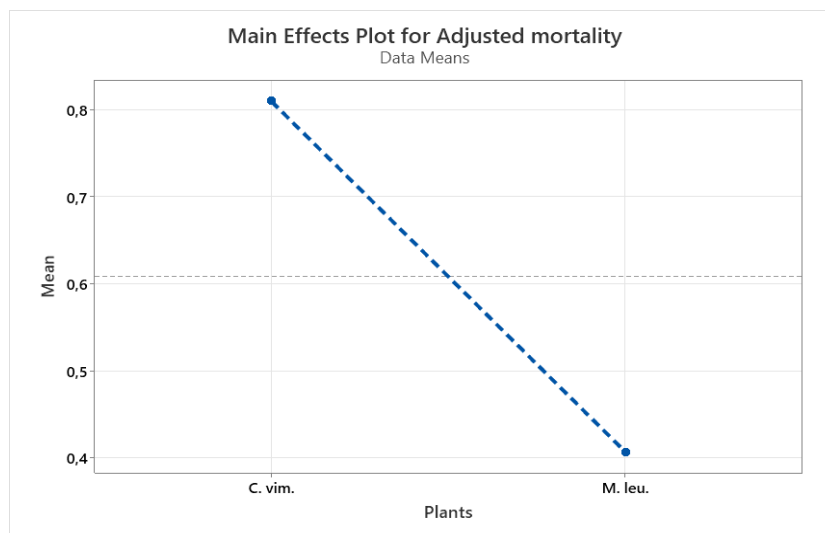
### Direct comparison of the two essential oils

*C. viminalis* is classified in Group A (average mortality = 81.0%) and *M. leucadendra* in Group B (40.7%). The absence of a common group confirms a statistically significant difference. *C. viminalis* oil is therefore almost

twice as effective as *M. leucadendra* oil against *S. zeamais*. This is also confirmed by the graph showing the main effects of the “Plant” factor and the “Dose × Plant” interactions.

**Table 9: Tukey’s tests: Plant factor (95% CI).**

| Plants                | N   | Mean   | Grouping |
|-----------------------|-----|--------|----------|
| <i>C. viminalis</i>   | 464 | 0,8098 | A        |
| <i>M. leucadendra</i> | 464 | 0,4068 | B        |



**Figure 4: Diagram showing the main effects of the “Plant” factor.**

The Tukey ranking of the “Plant × Dose” interaction (Table 10) and the “Plant × Dose” interaction plot reveal contrasting patterns between the two species. As a reminder, the order of the doses applied is as follows: D1 is the highest dose, D2 and D3 are intermediate doses, and D4 is the lowest dose.

For *C. viminalis*, the four doses form a homogeneous group A (0.803-0.812), indicating that even the lowest dose (D4) is sufficient to maintain high mortality (80%).

For *M. leucadendra*, the high doses D1-D2 remain effective (group A: 69-70%), whilst the low doses D3-D4 prove insufficient (group B: 7-16%), which corresponds to a normal increasing dose-response relationship.

**Table 10: Tukey's comparisons: Plant × Dose interaction (95% CI).**

| Plant*Dose            | N   | Mean   | Grouping |
|-----------------------|-----|--------|----------|
| <b>C. viminalis</b>   |     |        |          |
| D1                    | 116 | 0,8120 | A        |
| D2                    | 116 | 0,8120 | A        |
| D3                    | 116 | 0,8120 | A        |
| D4                    | 116 | 0,8034 | A        |
| <b>M. leucadendra</b> |     |        |          |
| D2                    | 116 | 0,7025 | A        |
| D1                    | 116 | 0,6937 | A        |
| D3                    | 116 | 0,1639 | B        |
| D4                    | 116 | 0,0673 | B        |

**Figure 5: Dose × Plant Interaction Diagram.**

## DISCUSSION

The yield of  $5,032 \pm 0,014\%$  obtained for *C. viminalis* from dried leaves is significantly higher than the values reported in the literature for this species. Fall et al. (2017)<sup>[10]</sup> reported a yield of 3.920% for dried (6-day) leaves of *C. viminalis* harvested from the botanical garden of the Faculty of Science and Technology in Dakar using hydrodistillation, which is the closest reference to our experimental conditions. Oyediji et al. (2009)<sup>[14]</sup>, on the other hand, obtained a lower yield (0.90%) from fresh leaves in South Africa.

Similarly, the yield of  $1,684 \pm 0,210\%$  obtained for *M. leucadendra* from dried leaves is higher than the values published in the literature for this species using hydrodistillation. Song et al. (2016)<sup>[15]</sup> cites a yield of 1.75% for hydrodistillation extractions from dried leaves of *M. leucadendra*, which is almost the same as the yield obtained here. Fall et al. (2017)<sup>[10]</sup> reported a yield of 1.277% for dried leaves (6 days) harvested in the Hann/Dakar Forest Park.

These variations in yield, observed for both species, can be explained by factors well documented in the literature on essential oils: the duration and conditions of drying, the phenological stage at harvest, the geographical origin and climatic conditions of the collection site, as well as

the operational parameters of hydrodistillation (duration, plant material to water ratio). The prolonged drying period (6 days) used by Fall et al. (2017)<sup>[10]</sup> generally leads to a relative concentration of volatile compounds through water loss, making this study the most comparable to our conditions.

The high, dose-independent insecticidal efficacy against *C. viminalis* (81% at all concentrations) can be explained by the synergy of its major compounds, whose mechanisms of action are well characterised in the literature on insecticidal essential oils.

Eugenol and meta-eugenol (10.10% combined) are recognized inhibitors of octopamine receptors, a neuromodulator system specific to arthropods and absent in vertebrates, which confers high toxicological selectivity on these molecules. This mechanism has been demonstrated by blocking the binding of octopamine to its receptors and by inhibiting the increase in cAMP normally induced by this neurotransmitter<sup>[16, 17]</sup>, as well as through pharmacological experiments using an octopaminergic receptor antagonist (phentolamine), which reversed the hyperactivity induced by eugenol in *Triatoma infestans*, in a manner comparable to that induced by octopamine itself.<sup>[18]</sup>

$\beta$ -caryophyllene (9.28%), a sesquiterpene with well-documented potent insecticidal activity, acts both through direct contact toxicity, with very low LD<sub>50</sub> values reported for several agricultural pests<sup>[19]</sup>, and through inhibition of acetylcholinesterase, as demonstrated in *Aedes aegypti* larvae exposed to the essential oil of *Piper tuberculatum*, of which it is the major constituent.<sup>[20]</sup> Its high affinity for lipid bilayers, demonstrated in vitro, also supports the hypothesis of an effect on neuronal membranes.<sup>[21]</sup>

$\alpha$ -Terpineol (9.39%) contributes to the lethal effect through a dual mechanism: disruption of the structure and permeability of biological membranes, demonstrated by electron microscopy on fungal membranes but applicable to the general membrane-targeting mode of action of monoterpenes<sup>[22]</sup>, and by inhibiting acetylcholinesterase, confirmed in vitro in *Bemisia tabaci* with IC<sub>50</sub> values in the millimolar range<sup>[23, 24]</sup>, as well as in a landmark study on the mechanism of AChE inhibition by monoterpenoids in stored-product insect pests.<sup>[25]</sup> The same compound has also been implicated, alongside eugenol, in the octopaminergic blockade responsible for the neurotoxic hyperactivity observed in the American cockroach.<sup>[17]</sup>

Phytol (12.21 %), the major diterpene, exhibits documented insecticidal and larvicidal activity against several species, notably termites, where its toxicity has been attributed to a cytotoxic action involving the invasion of cell membranes and disruption of the ionic balance, rather than to specific enzyme inhibition.<sup>[26]</sup> A high repellent activity of phytol has also been reported in *Anopheles gambiae* and *Aedes aegypti*<sup>[27]</sup>, which reinforces its role in the overall insecticidal effect observed.

The combined action of these compounds, which have complementary mechanisms of action (octopaminergic blockade, acetylcholinesterase inhibition and membrane disruption-explains the complete saturation of the lethal effect even at the lowest dose (D4), rendering higher doses statistically redundant.

The composition of *M. leucadendra* consists of 53% hydrocarbon sesquiterpenes, mainly  $\beta$ -humulene (24.97%) and aromadendrene (17.59%). These compounds exert insecticidal activity primarily through physicochemical mechanisms.  $\beta$ -Humulene, particularly as found in the essential oils of cinnamon and clove which are rich in it, has been linked to neurotoxic effects in insects, including paralysis and muscle contractions suggestive of a blockage of octopaminergic binding sites, as well as to oxidative toxicity caused by the overproduction of ROS and depletion of glutathione.<sup>[28]</sup> Aromadendrene, a tricyclic sesquiterpene, belongs to a family of compounds with documented insecticidal and cytotoxic properties found in plant essential oils, although its precise mechanism remains less well characterised than that of oxygenated

compounds.<sup>[29]</sup> This physicochemical contact action explains why its efficacy is proportional to the concentration applied.

$\alpha$ -Eudesmol (15.96%), an oxygenated sesquiterpene, is an inhibitor of P/Q-type calcium channels whose neuromodulator activity has been demonstrated on sensory nerve endings, where it blocks the depolarisation-induced release of neuropeptides.<sup>[30]</sup> Although this mechanism has been characterised in vertebrates, the relative conservation of voltage-dependent calcium channels across nervous systems supports the hypothesis of a neuroactive effect in arthropods, although its expression requires reaching a threshold concentration.

The observed increasing dose-response relationship (D1-D2 active in group A, D3-D4 inactive in group B) is therefore biologically consistent: high doses (D1, D2) reach the minimum effective concentration for these molecular targets, whilst low doses (D3, D4) remain sublethal. The absence of phenylpropanoids and the low proportion of oxygenated monoterpenes limit the spectrum of action of this extract compared with *C. viminalis*, whose efficacy is based on a multi-target synergy (octopaminergic, cholinergic and membrane-related) that is active even at the lowest doses.

## CONCLUSION

This comparative study assessed the bioinsecticidal potential of essential oils extracted from the dried leaves of two Myrtaceae species, *Callistemon viminalis* and *Melaleuca leucadendra*, against *Sitophilus zeamais*, a major pest of maize stores. Hydrodistillation revealed a significantly higher yield for *C. viminalis* (5,032  $\pm$  0,014%) compared with *M. leucadendra* (1,684  $\pm$  0,210%), whilst GC/MS analysis highlighted contrasting phytochemical profiles: an exclusive phenylpropanoid fraction (eugenol, meta-eugenol, methyleugenol) associated with an abundant diterpene phytol in *C. viminalis*, as opposed to a predominance of hydrocarbon sesquiterpenes ( $\beta$ -humulene, aromadendrene) in *M. leucadendra*.

Bioassays confirm that this chemical divergence results in different modes of biological action. *C. viminalis* oil exhibits total insecticidal activity (81% corrected mortality) that is dose-independent, with a lethal effect achieved within the first 24 hours and maintained regardless of the concentration tested (D1 to D4), reflecting rapid saturation of the toxic effect driven by the synergistic and multi-target action of its major compounds (octopaminergic blockade, acetylcholinesterase inhibition, membrane disruption). Conversely, *M. leucadendra* exhibits a classic, increasing dose-response relationship, with significantly lower efficacy (40.7% on average), limited to high doses (D1-D2: 69-70%) and virtually zero at lower doses (D3-D4: 7-16%), consistent with a mode of action that is primarily physicochemical and contact-based.

From a statistical perspective, the three-factor ANOVA confirms the highly significant nature of all main effects and their interactions, in particular the Plant effect ( $F = 154.84$ ;  $p < 0.001$ ), which unequivocally establishes the insecticidal superiority of *C. viminalis* over *M. leucadendra*, the former being nearly twice as effective.

In light of these results, *Callistemon viminalis* stands out as the most promising candidate for use in post-harvest protection of maize stocks against *S. zeamais*, owing to its high extraction yield, maximum efficacy even at low doses, and rapid lethal action, key advantages for low-cost practical application in smallholder farming contexts. This research paves the way for further avenues of investigation: the formulation of the essential oil for field application; the assessment of its persistence of action and stability in storage; the study of synergistic or antagonistic effects between its major compounds (eugenol,  $\beta$ -caryophyllene,  $\alpha$ -terpineol, phytol), and the extension of its spectrum of activity to other pests of stored foodstuffs.

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