# Fumigant toxicity of two Cineole-rich essential oils and their combinations for the control of *Carpophilus hemipterus* Linnaeus and *Sitophilus oryzae* Linnaeus

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**ملخص ـ السمية عن طريق التبخير لزيتين أساسيان غنيان بالسينول ومزيجهما كطريقة مكافحة *Carpophilus hemipterus* و *Sitophilus oryzae*.** ان الغرض من هذا العمل هو استكشاف النشاط الحشري عن طريق التبخير للزيوت الأساسية المستخرجة من نوعين من الأوكالبتوس: *Eucalyptus maidinii* F.Muell و *Eucalyptus cineria* F.Muell التي تم جمعها من غابات شوشة (سجنان) وسوينيات (عين دراهم). تم تقييم نجاعة التبخير باستخدام الزيوت الخام وحدها وأيضا بمزجهما مع بعضها. تم الحصول على الزيوت الأساسية باستخدام طريقة التقطير المائي. تم تحديد مركبات الزيوت من خلال تقنيات قياس الطيف الكتلي للغاز (GC و GC-MS). وقد لوحظ النشاط الحشري للزيوت (تقنية التبخير) ضد اثنين من الآفات الضارة بالمنتجات المخزنة: *Carpophilus hemipterus* و *Sitophilus oryzae*. أظهرت تحليلات GC و GC-MS أن 1،8 سينيول هو المركب الرئيسي المهيمن للزتين بقيم 72.85% و 69.04% لـ *E. cineria* و 85.49% و 68.99% ل *E.maidinii* جمعت على التوالي من شوشة, و سوينيات. علاوة على ذلك، لم يكن لتركيبة الزيوت الأساسية تأثير تآزري لأن قيم الجرعة النصفية القاتلة ($CL_{50}$) كانت إحصائيًا متكافئة مع أنشطة الزيوت المفردة. أظهرت النتائج اختلاف في فاعلية التبخير حسب التركيزات ومدة التعرض للزيوت. بدت زيوت *E. maidinii* أكثر فعالية ضد الحشرات وخاصة *C. hemipertus* بنسبة 98% من الوفيات التي تم الحصول عليها بعد 24 ساعة من التعرض بتركيز 74.73 ميكرولتر / لتر من الهواء. بشكل عام، أشار هذا العمل إلى جدوى استخدام الزيوت الأساسية الغنية بالسينيول لمكافحة آفات المنتجات المخزنة، في حين يجب إجراء المزيد من الأبحاث حول تأثيرها على جودة الأغذية.
**الأوكالبتوس/ 1،8 سينيول / *Carpophilus hemipterus* / *Sitophilus oryzae***

**Résumé - Toxicité par fumigation de deux huiles essentielles riches en Cinéole et de leurs combinaisons pour la lutte contre *Carpophilus hemipterus* Linnaeus et *Sitophilus oryzae* Linnaeus.** Le but de ce travail était d'explorer l'activité insecticide des huiles essentielles extraites de deux espèces d'Eucalyptus : *Eucalyptus maidinii* F.Muell. et *Eucalyptus cineria* F.Muell, collectées dans les arboretums de Choucha (Sejnene) et de Souiniet (Ain Draham). La toxicité des fumigeants a été évaluée pour les huiles essentielles seules et leurs combinaisons. Les huiles essentielles ont été obtenues par la méthode d'hydrodistillation. L'identification des composés des huiles a été réalisée par des techniques de chromatographie en phase gazeuse et de spectrométrie de masse (GC et GC-MS). Une activité insecticide (toxicité des fumigants) a été constatée contre deux principaux nuisibles des produits stockés: *Carpophilus hemipterus* et *Sitophilus oryzae*. Les analyses par GC et GC-MS ont montré que le 1, 8- cinéole était le composé principal dominant des huiles avec des valeurs de 72,85% et 69,04% pour *E. cineria* et 85,49% et 68,99% pour *E. maidinii* prélevées respectivement dans les forêts de Choucha et Souiniet. En outre, la combinaison d'huiles essentielles n'a pas eu d'effet synergique, car les valeurs de CL50 étaient équivalentes à celles des activités des huiles essentielles individuelles. Les résultats ont révélé que le potentiel fumigant variait en fonction des concentrations et de la durée d'exposition. Les huiles essentielles d'*E. maidinii* semblaient plus efficaces contre les insectes, en particulier contre *C. hemipertus*, avec une mortalité de 98% obtenue après 24 heures d'exposition à la concentration de 74,73 µl/ l d'air. Dans l'ensemble, ces travaux ont mis en évidence l'intérêt de l'utilisation des huiles essentielles riches en cinéol pour la lutte contre les ravageurs des denrées stockées, tandis que des études complémentaires sur l'impact des huiles essentielles sur la qualité des aliments devraient être entreprises.
***Eucalyptus* / 1, 8- cinéole / *Carpophilus hemipterus* / *Sitophilus oryzae***

**Abstract -** The purpose of this work was to explore the insecticidal activity of essential oils extracted from two *Eucalyptus* species: *Eucalyptus maidinii* F.Muell. and *Eucalyptus cineria* F.Muell collected from arboreta of Choucha (Sejnene) and Souiniet (AinDraham). Fumigant toxicity was assessed for essential oils alone and their combinations. Essential oils were obtained using hydrodistillation method. Identification of oils compounds was achieved through gas chromatography-mass spectrometry techniques (GC and GC-MS). Insecticidal activity (fumigant toxicity) was experienced against pests of stored products: *Carpophilus hemipterus* and *Sitophilus oryzae*. GC and GC-MS analyzes showed that 1, 8- cineole was the major compound of the oils with values of 72.85% and 69.04% for *E. cineria* and

85.49% and 68.99% for *E. maidinii* collected respectively from Choucha and Souiniet arboreta. Moreover, essential oils combination did not give a synergistic effect since the $LC_{50}$ values were equivalent with those of single oils activities. Results revealed that fumigant potential varied upon concentrations and exposure duration. *E. maidinii* essential oil seemed more effective against the two insects especially *C. hemipertus* with 98% mortality obtained after 24 h of exposure at the concentration74.73 µl/ l air. Overall, this work pointed out the interest of using rich-cineole essential oils for the control of stored-products pests, while, further investigations on the impact on food quality should be undertaken.
***Eucalyptus* / 1, 8- cineole / *Carpophilus hemipterus* / *Sitophilus oryzae***

## 1. INTRODUCTION

In Tunisia, cereal has been considered one of the most important components of Tunisian agriculture. In fact, they have a diversity of uses such as foods, in an assortment that includes the usage of different technological processing methods. Durum wheat is the major crop and the most widely cultivated cereal (Latiri *et al.*, 2010). As well as, in Tunisia, dates are considered one of the most important and valuable export commodity. According to Saleh (2020) the Tunisian date production reached 340.000 metric tons. The dates' production constitutes an essential part of the total agricultural outputs of the country. Furthermore, Tunisia is one of the world's biggest producers and exporters of dates (GIFruits, 2009). High significant infestations caused by *Sitophilus oryzae* (Linnaeus, 1763), that has been considered one of the most dangerous pests of cereal grains and their products (Baloch, 1992; Zakladnoi and Retanova 1987). Additionally, According to Blumberg (2008), dates are likely to be infested by insect pests during the storage period. *Carpophilus hemipterus* Linnaeus is one of the primary insect pests causing serious damage to dates (Alzadjali *et al.*, 2006). For a long time, farmers were aware of food preservation problems (Benayed, 2008). Despite all the precautions taken, such as preventives mesures stored products remain a supportive environment for the growth and development of many pests. Previous research demonstrated that coleopterans are of great economic importance damaging 10 to 15% of grains during postharvest (Madrid *et al.*, 1990; Kaur *et al.*, 2017). *Sitophilus oryzae* Linnaeus and *Carpophilus hemipterus* Linnaeus are considered among the most harmful pests in Tunisia (Jarraya, 2003) and other countries around the world (Baloch, 1992; Wrigeley *et al.*, 1994). Effective control methods are needed to reinforce the safe protection already existing of stored products (Ayvaz *et al.*, 2008). In many storage systems, chemical control

have been considered one of the most economical tool controlling and managing theses stored insect pests (Snelson, 1987; Azelmat *et al*., 2006).
Recently, chemical control is considered as a hazardous tool on environment and especially on human health (Bell, 2000). Therefore, alternative methods for control are strongly needed. In nature, various plants and their extracts (as essential oils) are recognized for their effectiveness against stored insect pests.
Essential oils are extracted from different parts of plants (Daferera *et al.*, 2003; Al-Hamwi *et al.,* 2011) and may be considered as suitable alternative to control stored insect pests and to preserve food products (Shaaya *et al*., 1997). According to Mohan and Ramsawamy (2007) essential oils are known by their insecticidal properties and characterized by their high volatile compounds with strong odor and lipophilic properties (Bakkali *et al.* 2008). Various biological properties were already allocated to the genus *Eucalyptus.* In Tunisia, 177 *Eucalyptus* species have been introduced and acclimatized in 30 arboretums since 1957 (Ahmed Serier, 2016). *Eucalyptus* oils are endowed with insecticidal activities due to components such as p-cymene, eucamalol, limonene, linalool, α-pinene, γ-terpene, α-terpineol, allocimene, aromadendene and 1,8-cineole (Yang *et al.,* 2004). Besides, Papachristos *et al.* (2004); Maciel *et al.* (2010) and Jemâa *et al.* (2012) indicated the presence of relationship between Eucalyptus essential oils and insecticidal activity. This activity is related to high amounts of 1,8-cineole and α-pinene. Furthermore, other essential oils compounds have before proven notable insecticidal activity like aromadendrene and α-terpineol (Prates *et al.,* 1998; Su *et al.,* 2006; Liu *et al.,* 2008). In the same context, Batish *et al.* (2006) pointed out that the two monoterpenes 1,8 cineole and α-pinene support the insecticidal toxicity of Eucalyptus oils aigainst insect pests of stored products. Similarly, Batish *et al.* (2008) cited that essential oils bioactivity varied upon their constituents and their individual compounds. On the other hand, Benazzedine (2010) and Tapondjou *et al.* (2003) have shown the effectiveness of *Eucalyptus saligna* (Sm, 1797) and *Chenopodium ambrosioides* (Mosyakin and Clemants, 2002) essential oil against *Callosobruchus maculatus* (Fabricius, 1775). The toxicity of *Eucalyptus* essential oils' was investigated on various species of beetles and demonstrated that insecticidal activity depend on the insect species and exposure time (Jemâa *et al.*, 2013).
The aim of this study was (i) to explore and compare the chemical constituents of two *Eucalyptus* species namely: *E. maidenii* and *E cineri* collected from two different arboreta in north Tunisia, (ii) to assess their fumigant toxicity alone and in combination against adults of two major insect pests of stored products *C. hemipterus* and *S. oryzae*.

## 2. MATERIAL AND METHODS

### 2.1.Insect rearing

Rearing of the two insect species *Sitophilus oryzae* and *Carpophilus hemipterus* was carried out in the Laboratory of Biotechnology Applied to Agriculture, Entomology section of the National Institute of Agricultural Research of Tunisia (INRAT). *Sitophilus oryzae* were reared on semolina and *C. hemipertus* was initiated from infested dates in plastic boxes. The rearing conditions were 25 ± 1 °C, a relative humidity of 65 ± 5% and a photoperiod of 12h Light/12h dark (Dal Bello *et al.,* 2000). The boxes were covered with muslin cloth and after two week s the original adults were removed by sieving. The boxes were observed daily to collect adults according to their age group. Newly emerged adults (7 days old) were used for the bioassays.

### 2.2. Plant material

Leaves of *E. cinerea* and *E. maidenii*, were collected from the arboreta of Souiniet, Ain Draham, Jendouba (2221 m 36°46'20" N8° 41'08'E) and Choucha, Sejnen, Bizerte (1455 m 37°03'19"N9°14'31'E) during one season (September 2019). Adult leaves from each *Eucalyptus* species were harvested from 10 plants from each site. Leaves were placed in the shade for drying for 7-10 days at room temperature. Subsequently, the dried leaves are cut into small pieces and prepare for extraction.

#### 2.2.1. Essential oil extraction and chemical analysis

Essential oils were extracted by hydrostillation of 150 g of dried leaves using a modified Clevenger-type apparatus for 4 h as described by (Jemâa *et al.*, 2012).

#### 2.2.2. Chemical analysis

Chemical analysis was performed at the National Institute of Applied Science and Technology, Department of Biology, Laboratory of Plant Biotechnology. Quantitative and qualitative analysis of the essential oils was performed using an Agilent-technologies CPG-SM. The GC analysis of the oils was carried out on a GC 7890 apparatus, equipped with a splits splitless injector attached to a HP column and a triple axis detector. Injector and detector temperature was 250°C, while, the column temperature was from 60 to 250 °C. The carrier gas flow rate He was 4 ml/min. A sample of 1µl was injected, using split mode (split ratio, 1:100). All quantification was determined through built-in handling program gives by the manufacturer of the gas chromatograph. The composition was reported as a relative percentage of the total peak area. The identification of the essential oil constituent was carried out by using a comparison of their retention times to n-alkenes, compared to published data and spectra of authentic compounds.

### 2.3. Fumigant toxicity bioassay

Fumigant toxicity of *E. maidenii, E. cineria* essential oils and their mixture *E. maidenii***E. cineria* at the ratio 1:1) against adults of *C. hemipertus* and *S. oryzae* were tested in Plexiglas bottle (41.9 ml), in which 10 adults were released. Essential oil was deposed on filter paper disks (Whatman N°1) cut into 1 cm diameter pieces (Hamdi *et al.* 2015). Filter papers were impregnated with different concentrations 0.5, 1, 1.5 and 2µl of each essential oil. These doses have been chosen according to previous work conducted in the same conditions (Jemâa *et al.*, 2012). Doses were converted to give equivalent fumigant concentrations of respectively 13.16, 26.31, 40.8, 52.64 µl/l air. The impregnated filter paper was then attached to the wall of the Plexiglas bottle which was closed hermetically. Each concentration and control was replicated five times. Mortality was followed each hour until the death of insects. Control insects (non-treated) were kept under the same conditions. The mortality was calculated using Abbot's correction formula (Abbott, 1925).

### 2.4. Statistical analysis

Statistical analysis was performed using SPSS statistical software version 20.0. All obtained values were the mean of five replications and were expressed as the mean ± standard error. Differences in values of each oil treatments were tested by ANOVA one way followed by Duncan test. Where necessary, data were transformed by squirt to meet the assumptions of normality. In addition, statistical analysis was conducted to estimate median lethal concentration ($LC_{50}$) and median lethal time ($LT_{50}$).The Pearson correlation coefficient using the test "r" Bravais Pearson was calculated for the interaction between 1,8-cineole percentage and mortality percentage.

## 3. RESULTS

### 3.1. Chemical composition of essential oils

Mean levels of the main chemical classes, others constituents and total identified compounds of the essential oils were reported in Table 1, while the proportions of major compounds were shown in Figure 1. Results demonstrated that monoterpens were present in high levels for all studied oils. Some compounds were common to different oils but in variable percentages. *E. maidenii* and *E. cineria* oils were distinguished by low proportions of sesquiterpene. The major component of *E. cineria* essential oil was 1,8-cineole with respective values of 72.85% (Choucha arboretum) and 69.04% (Souiniet arboretum) followed ɒ-pinene (4.45%) and (6.99%) respectivelt for Choucha and Souiniet arboretum. Besides, concerning *E. maidenii* essential oil, 1,8-cineole was also the major compound with percentages varying between 68.99% (Souiniet arboretum) and 85.49% (Choucha arboretum) followed by Globulol (2.3%) and α-terpineol (7.04%) respectively for Choucha and Souiniet arboretum ɒ-pinene (6.5%) (Table 1, Figure 1).

Table 1: Chemical composition, others compounds and total identified constituents (%) of essential oils extracted from *Eucalyptus cinerea* F.Muell. and *Eucalyptus maidenii* F.Muell. collected from the arboreta of Choucha and Souiniet (North Tunisia).

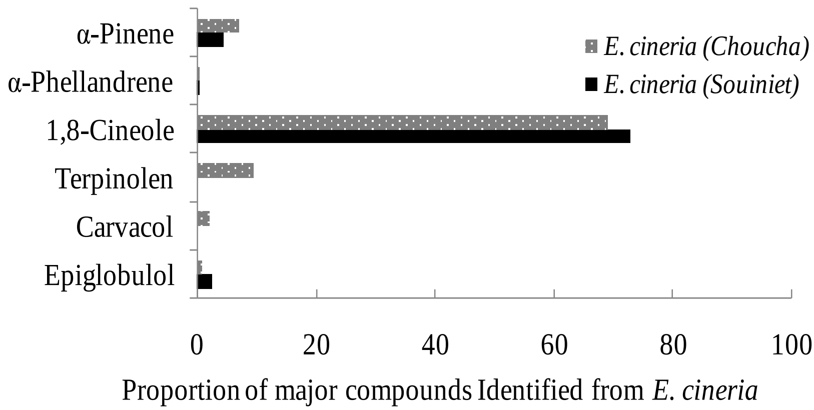
| **N°** | **Compounds** | ***E. cinerea*** **(%)** | | **RI** | **E. maidenii (%)** | | **RI** |
|---|---|---|---|---|---|---|---|
| | | **Choucha** | **Souiniet** | | **Choucha** | **Souiniet** | |
| | | **Monoterpene hydrocarbons** | | | | | |
| | | **14.04** | **8.55** | | **1.3** | **8.82** | |
| **1** | α–Pinene | 4.45 | 6.99 | 5.52 | 1.3 | 6.5 | 5.52 |
| **2** | β-pinene | - | - | | - | - | - |
| **3** | β-myrcene | - | | | - | - | - |
| **4** | Dl-camphene | 0.22 | - | 5.87 | - | - | - |
| **5** | Isovalerate | - | - | | - | 0.55 | 10.16 |
| **6** | α–phellandrene | 0.23 | 0.39 | 7.26 | | | |
| **7** | γ–Terpinene | - | 0.2 | 8.79 | - | 0.18 | 8.8 |
| **8** | Trans-pinocarveol | 1.57 | 0.97 | 11.37 | - | - | - |
| **9** | Carene | 6.61 | - | 18.15 | - | - | - |
| **10** | Spathulenol | 0.96 | - | 25.27 | - | - | - |
| | | **Oxygenated Monoterpenes** | | | | | |
| | | **78.15** | **86.55** | | **87.89** | **77.83** | |
| **12** | β-cymene | 1.38 | 1.36 | 7.84 | 1.15 | - | 7.84 |
| **13** | 1.8-cineole | 72.8 | 69.04 | 8.07 | 85.49 | 68.99 | 8.07 |
| **14** | Trans-pinocarveol | - | - | - | _ | 1.05 | 11.39 |
| **15** | Trans-pinocarvone | - | - | - | _ | 0.23 | 12.1 |
| **16** | Borneol | 0.76 | - | 12.27 | - | - | - |
| **1** | Terpinene-4-ol | 0.28 | 0.6 | 12.6 | _ | 0.52 | 12.62 |
| **18** | α–terpineol | 2.66 | 3.83 | 13.08 | 1.03 | 7.04 | 13.06 |
| **19** | Cis-carveol | 0.22 | - | 13.98 | 0.22 | _ | 13.98 |
| **20** | Carvacrol | - | 2.04 | 16.9 | - | - | - |
| **21** | Methylgeranate | _ | 0.31 | 17.31 | - | - | - |
| **22** | α–terpinolen | _ | 9.37 | 18.16 | - | - | - |
| | | **Sesquiterpenes hydrocarbons** | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | **0.55** | **1.54** | | **0.95** | **3.85** | |
| **23** | β-caryophyllene | 0.24 | 0.26 | 20.44 | | | |
| **24** | Aromadendrene | _ | 0.25 | 21.06 | 0.95 | 3.16 | 20.11 |
| **25** | Bicyclogermacrene | 0.31 | _ | 22.80 | - | - | - |
| **26** | γ-gurjunene | - | 1.03 | 25.47 | - | - | - |
| **27** | α– gurjunene | - | - | - | - | 0.29 | 20.83 |
| **28** | Aristolene | - | - | - | - | 0.19 | 21.04 |
| **29** | Ledene | - | - | - | - | 0.21 | 22.77 |
| | | **Oxygened Sesquiterpenes** | | | | | |
| | | **2.58** | **0.66** | | **2.77** | **7.21** | |
| **30** | Epiglobulol | 2.58 | 0.66 | 25.4 | 0.71 | 2,14 | 24.73 |
| **31** | Globulol | | | | 2.06 | 5.07 | 25.45 |
| | | **Others compounds** | | | | | |
| | | **13.73** | **0** | | **0** | **0.27** | |
| **32** | Isooctane | 0.19 | _ | 3.16 | - | - | - |
| **33** | α–fenchylalcohol | 0.27 | _ | 10.57 | - | - | - |
| **34** | Eremophilene | - | - | - | 0 | 0.27 | 26.33 |
| | | **Minor-compounds** | | | | | |
| | | **4.21** | **2.7** | | **7** | **3.07** | |
| **% Total identified compounds** | | **99.99** | **100** | | **99.91** | **100** | |

RI: Retention Index

## 3.2. Mortality rate

Mortality rates of *C. hemipterus* and *S. oryzae* exposed to essential oils during 24 h were shown in Figure 2. Results revealed that higher mortality rates were obtained with increasing concentrations of essential oils. Significant differences have been observed between *E. cineria* and *E. maiden*ii essential oils on *C. hemipterus* collected from Souiniet (F=20.36, p≤0.00), and on *S. oryzae collected* [from Choucha (F=13.83, p≤0.00); Souiniet (F=11.26, p≤0.001)]. For *C. hemipterus,* mortality rates reached their maximum values when adults are exposed to *E. maiden*ii collected respectively from Choucha (98%) and Souiniet (88.5%) at the concentration 74.73 µl/ l air. Indeed, for *S. oryzae,* mortality values were high at the concentration 74.73 µl/ l air and reached 77% for *E. cineria* oil collected from Choucha and 40% for *E. maiden*ii collected from Souiniet. Comparaison between essential oils collected from the same arboretum revealed that *E. maiden*ii oil was more toxic against *C. hemipterus* adults [Choucha (df=1, F=5.92, p≤0.006); Souiniet (df=1, F=5.93, p≤0.006)]. However, *E. maiden*ii oil colleted from Souiniet was more toxic against *S. oryzae* (df=1, F=8.17, p≤0.001). Furthermore, among the tested insects, no significant differences were observed regarding the impact of essential oils collected from the two arboreta Choucha and Souiniet [*E. maiden*ii (df=1, F=0.05, p≤0.81); *E.cineria* (F=1.84, p≤0.18) and *E. maiden*ii **E. cineria* (df=1, F=2.58, p≤0.11)].



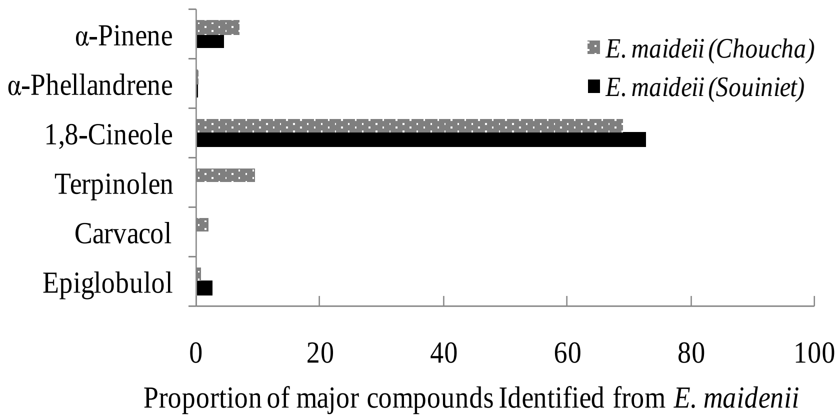




Figure 1: Proportion of major compounds identified from leaves of ten plants of *Eucalyptus cineria* F.Muell. and *Eucalytus maidenii* F.Muell. collected from Souiniet and Choucha arboreta.

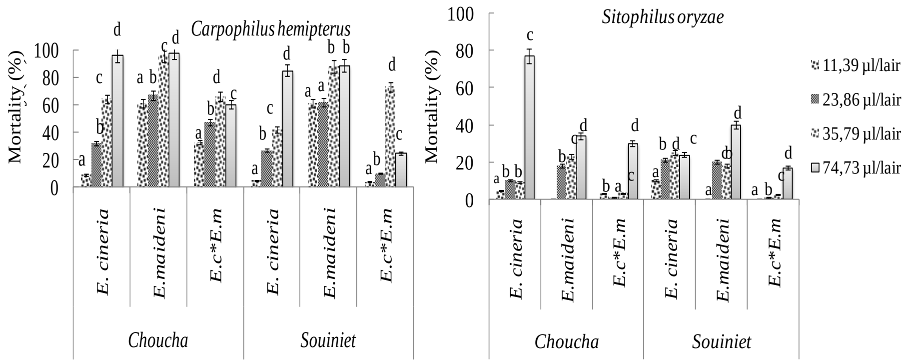



Figure 2: Efficacy of *Eucalyptus cineria* F.Muell. and *Eucalytus maidenii* F.Muell. essential oils against *Carpophilus hemipterus* (n=50) and *Sitophilus oryzae* (n=50) after 24 h of exposure time. Letters above bars indicate significant differences between concentrations. Bars with the same letter are not significantly different at $p<0.05$.

### 3.3. Toxicity variation in $LC_{50}$ values

Table 2 illustrated results of $LC_{50}$ values obtained for *E. cineria* and *E. maiden*ii essential oils collected from the two arboreta Choucha and Souiniet on *C. hemipterus* and *S. oryzae* after 24 h of exposure. Results demonstrated that *C. hemipterus* was more susceptible to essential oils of *E. maidenii* and *E. cineria* as compared to *S. oryzae*. Furthermore, according to the data reported in Table 1, *E. maidenii* exhibited the highest toxicity against *C. hemipterus* followed by *S. oryzae*. Besides, $LC_{50}$ values of *E. maidenii* collected from Choucha arboretum showed that this oil possessed stronger fumigant toxicity as compared to the $LC_{50}$ value of *E. cineria* collected from Souiniet arboretum (Table 2). However, *S. oryzae* presented the highest $LC_{50}$ values at the concentrations 92.13 µl/ l air and 176.16 µl/ l air respectively after exposure to *E. cineria* collected from Choucha and Souiniet arboreta.

### 3.4.Toxicity variation in $LT_{50}$ values

Table 3 reported results of Lethal Time ($LT_{50}$) calculated for adults of *C. hemipertus* and *S. oryzae* exposed to *E. cineria* and *E. maiden*ii essential oils. Results revealed that essential oils extracted from plants collected from two arboreta were more toxic toward *C. hemipterus* adults (Table 3). Moreover, results indicated that essential oils extracted from *E. maidenii* were more toxic against the two insects followed by the combination *E. maidenii***E. cineria*. The $LT_{50}$ values of essential oils collected from Choucha arboretum against *S. oryzae* at the concentration 74.73 µl/ l air were respectively 30.83 h for *E. maidenii*, 35.87 h for *E. cineria* and 63.51 h for the combination *E. maidenii* E. cineria*. Thus, *S. oryzae* was the less susceptible as compared to *C. hemipterus*. Nevertheless, essential oils extracted from plants collected from Souiniet arboretum were more toxic against *S. oryzae* followed by *C. hemipterus* (Table 3).

Table 2: $LC_{50}$ values of essential oils extracted from *Eucalyptus cineria* F.Muell. and *Eucalytus maidenii* F.Muell. collected from the two arboreta Choucha and Souiniet against *Carpophilus hemipterus* (n=50) and *Sitophilus oryzae* (n=50) after 24 h of exposure.

| Arboretum | Oils | Insects | $LC_{50}$[1] | $\chi^2$ | Slope±SE[2] |
|---|---|---|---|---|---|
| **Choucha** | *Eucalyptus cineria* | *C.hemipertus* | 33.17 | 7.56 | 0.05±0.005 |
| | | *S. oryzae* | 58.88 | 7.51 | 0.04±0.003 |
| | *Eucalyptus maiden*ii | *C.hemipertus* | 3.96 | 14.2 | 0.03±0.006 |
| | | *S. oryzae* | 92.13 | 16.1 | 0.017±0.003 |
| | *Eucalyptus cineria** | *C.hemipertus* | 32.3 | 13.4 | 0.009±0.003 |
| | *Eucalyptus maiden*ii | *S. oryzae* | 65.12 | 5.45.4 | 0.03±0.004 |
| **Souiniet** | *Eucalyptus cineria* | *C.hemipertus* | 44.28 | 6.2 | 0.03±0.003 |
| | | *S. oryzae* | 176.16 | 5.50 | 0.006±0.003 |
| | *Eucalyptus maiden*ii | *C. hemipertus* | 7.76 | 11.47 | 0.01±0.003 |
| | | *S. oryzae* | 83.97 | 15.66 | 0.02±0.003 |
| | *Eucalyptus cineria** | *C. hemipertus* | 100.43 | 142.15 | 0.09±0.003 |
| | *Eucalyptus maiden*ii | *S. oryzae* | 108.15 | 0.44 | 0.02±0.006 |

Data tested by $\chi 2$-test for homogeneity of 1:1 ratio; 1. medianlethal concentration; 2. Standard error.

### 3.5. Correlation between mortality (%) and 1, 8-cineole percentage

Results reported that correlations between mortality (%) and 1,8-cineole percentage were high significant and positive relationships in the case of *C. hemipterus* (r=0.46, P≤0.01). Nevertheless, no significant correlations were obtained between mortality and 1, 8-cineolproportion for *S. oryzae* (r = -0.06, P≤0.6).

Table 3: $LT_{50}$ values of *Eucalyptus cineria* F.Muell. and *Eucalytus maidenii* F.Muell. collected from the two arboreta Choucha and Souiniet against *Carpophilus hemipterus* (n=50) and *Sitophilus oryzae* (n=50) for four concentrations.

| Site | Insects | Concentration (µl/lair) | $LT_{50}$[1] | | |
|---|---|---|---|---|---|
| | | | *E.cineria* | *E. maiden*ii | *E. cineria * E. maiden*ii |
| Choucha | *C. hemipertus* | 11.39 | 101.65 | 58.72 | 70.85 |
| | | 23.46 | 81.07 | 43.10 | 77.42 |
| | | 35.79 | 42.89 | 6.53 | 61.10 |
| | | 74.73 | 4.32 | 19.45 | 54.00 |
| | *S. oryzae* | 11.39 | 102.87 | 94.85 | 119.37 |
| | | 23.46 | 54.57 | 104.85 | 139.04 |
| | | 35.79 | 35.37 | 87.49 | 131.36 |
| | | 74.73 | 30.83 | 35.87 | 63.51 |
| Souiniet | *C. hemipertus* | 11.39 | 98.62 | 45.96 | 82.13 |
| | | 23.46 | 76.96 | 26.49 | 72.78 |
| | | 35.79 | 63.96 | 17.28 | 31.37 |
| | | 74.73 | 15.54 | 15.66 | 40.62 |
| | *S. oryzae* | 11.39 | 149.23 | 157.64 | 207.36 |
| | | 23.46 | 56.97 | 107.78 | 141.80 |
| | | 35.79 | 36.13 | 98.13 | 132.28 |
| | | 74.73 | 36.90 | 51.53 | 68.22 |

Data tested by $\chi 2$-test for homogeneity of 1:1 ratio; 1. median lethal time.

## 4. DISCUSSION

In the present study, the essential oils extracted from *Eucalyptus cineria* and *E.maidenii* showed effective toxicity against adults of *C. hemipterus* and *S. oryzae*. Results demonstrated that *E. maidenii* oil was more toxic against the two insect pests. However the combination of *E. cineria*E. maidenii* essential oils did not exhibit high fumigant toxicity as compared to separated oils. *S. oryzae* were more tolerant against these oils as the required concentrations to kill these adults were high than those required to *C.*

*hemipterus*. Our results and those discussed in previous studies revealed that the insecticidal activity of essential oils vary depending geographical origin, the location and plant species that can influenced the chemical composition of essential oils (Hussain *et al.*, 2008). Furthermore, earlier Ayoub and Elhassn (2014) reported that chemical characteristics differed from location to another. According to Yang *et al.* (2010) monoterpenes are the main constituents of many essential oils and owing to their elevated volatility they exhibited a high effectiveness for controlling insects of stored products. In the present research, we demonstrated that the toxic effect of the two *Eucalyptus* species may possibly be attributed to major compounds like 1, 8-cineole. Furthermore, the difference in efficacy between these oils could be attributed to different proportions of chemical components, that showed high percentage of oxygenated monoterpenes counting 1, 8-cineole. According to Lee *et al.*(2003) fumigant assay with 1, 8-cineole displayed 100% mortality. Among major components of *E. cineria* and *E. maidenii* essential oils, 1,8-cineole seemed to be more efficient against adults of *C. hemipterus* and *S. oryzae*, that showed 98% and 77% mortality at the concentration 74.73 µl/ l air. The high toxicity of 1,8-cineole was reported by earlier work against *Ephestia kuehniella, Ephestia cautella* and *Ectomyelois ceratoniae* (Jemâa *et al.,* 2012). Due to the oxygened monoterpenes, *E. maidenii* can exhibit an effective control against *C. hemipterus*. 1, 8-cineole seemed less performer against *S. Oryzae* with $LC_{50}$ value of 92.13µl/ l air. Moreover, no significant correlations have been observed between this major compound and mortality percentage of adults.

## 5. CONCLUSION

To conclude, the observed insecticidal activity demonstrated that essential oils are sources of biologically active vapors that provide an effective control against insects. The higher fumigant activity observed in this work was supported by richessment in 1,8-cineole on the essential oils. However, essential oils are adequately volatile to be removed by aeration and not frequently absorbed by grain when applied to food package (Choi *et al.*, 2017). Consequently, new technology as microencapsulation that is a technique of packing particles into continuous matrix shells (Qiu et al., 2017) are needed.

## ACKNOWLEDGMENTS

Authors are thankful to the Ministry of Agriculture of Tunisia for providing arboreta, and other facilities. This work was supported by funds from Laboratory of Biotechnology Applied to Agriculture, INRAT code LR11INRAT06 (Ministry of Higher Education and Scientific Research of Tunisia).

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