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# Fumigant Toxicity of *Eucalyptus camaldulensis* and *Callistemon viminalis* Essential Oils on *Sitophilus granarius*

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**Abstract** In this research insecticidal effects of *Eucalyptus camaldulensis* Dehnh and *Callistemon viminalis* (Gaertn) essential oils were investigated against adults (1-3 days old) of *Sitophilus granarius* L. in 280 ml glass vials with or without wheat grains. The essential oils were obtained by hydrodistillation method, using a modified Clevenger type apparatus. Experiments were carried out at 27±1°C and 65±5% R.H. under dark conditions. Results of fumigant toxicity tests showed that the mortality was increased with increase in concentration and time. Lethal concentration of *E. camaldulnsis* and *C. viminalis* to 50% of adults ($LC_{50}$) was estimated to be 18.22 and 9.73 µL/L air, respectively. Lethal time to kill 50% of adults ($LT_{50}$) at concentrations 35.71, 32.14, 28.57 and 25 µL/L air were lasted 18.00, 17.08, 16.55, and 16.09 h for *E. camaldulnsis* oil and 15.67, 14.89, 14.51, and 14.04 h for *C. viminalis*, respectively. The most sensitive stage to the essential oils was egg of *S. granarius* and that decreased with Growth gradualist. The activity of essential oils decreased significantly in jars filled with wheat kernels. $LC_{50}$ values of *E. camaldulnsis* and *C. viminalis* to kill *S. granarius* adults were 647.32 µL/L air and 629.34 µL/L air, respectively; showing the low fumigant efficacy of the oil in the grain storage. The cause may be due to a considerable sorption of essential oil by wheat grains and insufficient permeability of the oil vapors into seed inter-space.

**Keywords:** Fumigant toxicity, *Sitophilus granarius*, *Eucalyptus camaldulensis*, *Callistemon viminalis*

## Introduction

Essential oils from plants are valuable secondary metabolites which have already been used as raw materials in many fields, including perfumes, cosmetics, phytotherapy, and nutrition. These oils also offer potential as sources of insecticides with environmental compatibility (Katz *et al*., 2008). Eucalyptus and Butlbrush (family Myrtaceae), an Australian native, represented by around 700 species is a genus of tall, evergreen and magnificent trees cultivated world over for its oil, gum, pulp, timber, medicine and aesthetic

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value. Among the various wood and non-wood products, essential oil found in its foliage is the most important one and finds extensive use in food, perfumery and pharmaceutical industry (Batish *et al*., 2008). Recently, many studies have focused on the possibility of using plant essential oils for application to stored grain to control insect pests (Collins, 2006; De Carvalho and Da Fonseca, 2006). Methyl bromide (MeBr) and phosphine (PH3) are widely used fumigants. Methyl bromide has been used as a fumigant for at least 70 years and phosphine is used on over 70% of stored grain (Mueller, 1990). This wide role of MeBr and PH3 was restricted Under the Clean Air Act and Montreal Protocol, the use of MeBr was restricted, due to its potential ozone depleting properties. (World Meteorological Organization, 1995). Phosphine fumigation, may become limited in use due to increasing resistance of stored-grain insects to this material. (Bell and Wilson, 1995; Daglish and Collins, 1999). Different types of aromatic plant preparations such as powders, solvent extracts, essential oils and whole plants are being investigated for their insecticidal activity including their action as repellents, anti-feedants and insect growth regulators (Prakash and Rao, 1997; Isman, 2000; Weaver and Subramanyam, 2000). Weaver and Subramanyam (2000) suggested that fumigant activity in botanicals could have a greater potential use than grain protect in future on the basis of their efficacy, economic value and use in large-scale storages. More current research showed that essential oils and their constituents may have potential as alternative compounds to currently used fumigants (Singh *et al.*, 1989; Shaaya *et al*., 1991, 1997; Regnault-Roger *et al*., 1993; Dunkel and Sears, 1998; Huang and Ho, 1998; Huang *et al*., 2000; Tun *et al.*, 2000; Lee *et al*., 2001a, b). Lee *et al*. (2004) investigated toxicity of 42 essential oils extracted from seven Australian Myrtaceae genera (Eucalyptus, Melaleuca, Callistemon, Leptospermum, Kunzea, Baeckea and Angophora) was tested against the 3 major stored-grain insects: *S. oryzae*, *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.) also Rozman *et al*. (2009) researched aimed to determine the effect of wheat grain mass on the effectiveness of the essential oil cineole against *T. castaneumn*, *R. dominica* and *Cryptolestes ferrugineus* (L.) in an empty space, 50% and 95% spaces in occupied with wheat. Magalis *et al*. (2008) studied to evaluate the insecticidal effect of essential oils from *Gomortega keule* Baill, *Laurelia sempervirens* Tul., *Origanum vulgare* Oregano, *Eucalyptus globules* Labill, and *Thymus vulgaris* L. plants against *Sitophilus zeamais* Motschulsky and *Acanthophilus obtectus* Say. under laboratory conditions. Negahban and Moharramipoure (2007) studied fumigant toxicity of *Eucalyptus intertexa* R.T.Baker, *Eucalyptus sargentii* Maiden and *E. camaldulensis* against *Callosobruchus maculatus* Fab., *S. oryzae* and *T. castaneum*. Rice weevils are one of the major stored-grain pests' worldwide (Sinha and Watters, 1985).

Objectives: Therefore in these study insecticidal effects of *E. camaldulensis* and *C. viminalis* essential oils were investigated against *S. granarius* with or without wheat grains.

## Materials and methods

### *Insect rearing*

Initial population of *S. granarius* introduced on whole wheat and cultures were maintained in the dark in growth chamber set at 27 ± 1ºC and 65 ± 5% r.h. After three generations of rearing insects, all of adult insects were removed out; then emerged insects during 1-3 days were used for fumigant toxicity.

### *Plant materials*

Aerial parts of *E. camaldulensis* and *C. viminalis* were collected at full flowering stage in September 2009 from Sistan and Namak abrood province, respectively in Iran. Plants were identified by the Research Institute of Forests and Rangelands, Tehran, Iran. The plant materials were dried naturally on laboratory benches at room temperature with suitable ventilation in the dark conditions until crisp. The dried material was stored in paper pockets until needed.

### *Extraction of essential oils*

Plant materials were milled into fine powder using a milling machine. Fifty grams of the plant samples as well as 600 ml distilled water was put in a round bottom flask. Plant materials were subjected to hydrodistillation for 4 h using a modified Clevenger-type apparatus. The water of essential oils was removed using anhydrous sodium sulfate and the oils were stored in glass tubes at 4 ºC in refrigerator, until they were used. The *E. camaldulensis* and *C. viminalis* oils yielded 1.89 and 0.78% w/w, respectively on a dry weight basis.

## Fumigant toxicity

### *Assess 50% lethal Concentration ($LC_{50}$) without wheat grain*

Pre-experiments were conducted to assess the mortality among 20 until 80 percents of affected insects (Robertson *et al.*, 2007). Filter papers (2 cm diameter) was attached to the under surface of the screw cap of a glass vial volume 280 ml. Filter papers were impregnated with oils at different concentrations to release the fumigant. Then the caps were screwed tightly on the vial containing 10 adults (1–3 days old) of insect. Each concentration and control was replicated five times. Mortality was determined after 24 h from commencement of exposure. When no leg or antennal movements were observed, insects were considered dead. These symptoms were proved to ensure ultimate irrecoverable death in each period of exposure time. S. granarius were exposed to the essential oil of *E. camaldulensis* at 12.5, 14.28, 16.07, 17.85, 19.64, 23, 21, 25 and 28.58 μL/L air and for *C. viminalis* the concentrations were 3.57, 5.35, 7.14, 8.92, 10.71, 12.50, 14.28 and 17.85 mL/L air. Control insects were kept without any essential oil. Data obtained from each dose-response bioassay were subjected to probit analysis (Finney, 1971) to estimate $LC_{50}$ and $LC_{95}$ using SAS 6.12 (SAS Institute 1996).

### *Assess 50% lethal time ($LT_{50}$) without wheat grain*

Adults of *S. granarius* were exposed to each essential oil at concentrations of 25.00, 28.57, 32.14 and 35.71 mL/L air for 20 and 80 percent of affected insect that they dead after different times. Mortality for each concentration and exposure time was checked. Each test was replicated five times. Insects were presumed dead if they remained immobile and no leg or antennal movements were observed. Control insects were kept without any essential oil. Data obtained from each time-response bioassay were subjected to probit analysis (Finney, 1971) to estimate $LT_{50}$ and $LT_{90}$ values using SAS 6.12 (SAS Institute 1996).

### *Assess 50% lethal Concentration ($LC_{50}$) in wheat grain*

Fumigation bioassays with wheat were similar those without grain except the vials contained 94 g of wheat with 8% moisture content, which gave a 40% filling ratio (based on bulk density). Each vial containing 20 adults (1–3 days old) of *S. granarius*. Essential oils injected on the filter paper attached to the under surface of the screw cap of a glass. Mortality was determined after 24 h treatment at 27 ± 1 ºC. Various injection concentrations were tested and these treatments were replicated three times. Adults of *S. granarius* were exposed to the essential oil of *E. camaldulensis* at 535.71, 714.28, 892.85, 1178.57, 1535.71 and 1964.28 μL/L air and *C. viminalis* at 178.57, 285.71, 464.28, 714.28, 1142.85 and 1785.71 μL/L air concentrations was applied. Control insects were kept without any essential oil. After 24 h treatment, the vial was opened and the grain and insects separated. Dead and living insects were counted and were put in new vials with fresh wheat (50 g). Mortality was then reassessed but no significant difference was observed between the 24 and 48 h counts. Probit analysis and $LC_{50}$ and $LC_{95}$ values were calculated as mentioned before.

### *Effect of the essential oils on hidden eggs and immature stages*

The effect of essential oils of *E. camaldulensis* and *C. viminalis* on the development of egg and immature stages of *S. granarius* inside wheat kernels was also assessed. Batches of 94 g of wheat in 280 ml glass jars were infested with 30 unsexed adults to allow egg laying. The parent adults were removed after 7 days. One day after adult removal four batches of the grain were treated with essential oils at concentrations 714.28 and 1428.57 mL/L air. Control treatments were without any essential oils. Thereafter these treatments were repeated at 1, 2 and 3 weeks after removal. Then emerging adults were counted in treatment batches for a seven-weeks period, following the removal of adults. The data of adults percentage mortality and number of adults that were emerged at the different development stages were transformed to Arc sine $\sqrt{\frac{X}{100}}$ One way analysis of Variance was performed to compare the effects of the essential oils on adults emersion (Tukey, $P< .05$).

## Results

### *Assess 50% lethal Concentration ($LC_{50}$)*

*E. camaldulensis* and *C. viminalis* essential oils showed strong fumigant activity against *S. oryzae* adults in empty jars. In Table 1, the results showed that *S. granarius* adults were comparatively more susceptible to *C. viminalis* ($LC_{50}$=9.73 µl/l air) than *E. camaldulensis* oil ($LC_{50}$ =18.21 µl/l air) (no overlap in 95% fiducial limits ). Relative median potency of *C. vimimalis* oil versus *E. camaldulensis* oil was significant, confirming the more tolerance of the *S. granarius* to *E. camaldulensis* than *C. viminalis*.

### *Assess 50% lethal time ($LT_{50}$)*

In all cases, a strong difference in mortality of the insect was observed as oil concentration and exposure time was increased. $LT_{50}$ was obtained at concentrations of 25.00, 28.57, 32.14 and 35.71 µL/L air. For *E. camaldulensis*

$LT_{50}$ values were 18.00, 17.08, 16.55 and 16.09 h and for *C. viminalis* 15.67, 14.89, 14.51 and 14.04 h was obtained respectively. Fig. 1 and Table 2 show that the essential oil of *C. viminalis* is more toxic than *E. camaldulensis*.

### *Assess 50% lethal Concentration ($LC_{50}$) in grain*

Table 3, show that $LC_{50}$ values of *E. camaldulensis* and *C. viminalis* in grain was 647.32 μL/L air and 629.34 μL/L air, respectively that haven't caused significantly on insects (overlap in 95% fiducially limits). Essential oils when applied in grain were less effective against *S. granarius* than when applied in empty jars. Therefore, high concentrations of essential oils are needed for treated insects.

### *Effect on hidden eggs and immature stages in grain*

Adult emergence from wheat pre infested with egg, early larvae and late larvae stages of *S. oryzae* was significantly reduced by essential oils. Concentration of 714.28 μL/L air significantly reduced emergence of the adult from treated egg (72.50 %), early larvae (49.40 %) and late larvae (39.00 %) for *E. camaldulensis* essential oil. For *C. viminalis* oil at same concentration obtained; reduction in adult emergence from egg, early and late larvae were 82.86%, 47.88% and 52.30%, respectively (Fig.2). Applied concentration 1428.57 μL/L air of both oils completely inhibited the development of egg instars of *S. oryzae* and significantly reduce emergence of adult insect of early larvae (6.25 %) and late larvae (9.09 %) for essential oil of *E. camaldulensis* and for *C. viminalis* oil the reduction in emergence were 3.78 % and 7.38 % respectively.

## Discussion

The essential oil extracted from *C. viminalis* was more effective than essential oil of *E. camaldulensis* on *S. granarius*. The major components obtained by GC-MS analysis of *E. camaldulensis* oil were 1,8-cineole (26.1 %), a-pinene (12.6 %) and β-phellandrene (12.26%) (Sefidkon *et al*,. 2006) 1,8-cineole (61.7 %), a-pinene (24.2 %), Methyl acetate(5.3%) were identified as major components in the essential oil of *C. viminalis* ( Srivastava *et al*., 2003). Among the various components of eucalyptus oil, 1,8-cineole is the most important one and, in fact, a characteristic compound of the genus Eucalyptus, and is largely responsible for a variety of its pesticide properties (Duke, 2004). Negahban and Moharramipour, (2007) reported the *E. camaldulensis* oil had fumigant toxicity to *S. oryzae* and they obtained $LC_{50}$ 12.06 μL/L Air after 24 h exposure time to the oil. It may be concluded from their results that *S. oryzae* is more susceptible than *S. granaries*. 100% mortality was observed on *S. garanarius* when exposed for 24h to *Carum copticum* L. and *Cuminum cyminum* L. (Ziaee, 2104). Lee *et al.* (2004) were investigated fumigant toxicity of six essential oils with and without wheat against on *S. oryzae*. Essential oil of *Callistemon sieberi* were 3 to 4 times less toxic (in terms of percentage of insects killed) with wheat at a 50% filling ratio and up to 9 times in the case of *Eucalyptus codonocarpa*, compared with that without wheat. Rozman *et al.* (2009) determined bioactivity of cineole against *S. oryzae* on stored wheat in spaces differently filled up with wheat (empty space, 50% and 95% filled up). Concentration of 50 g m-3 cineole in empty space induced nearly 100% mortality of *S. oryzae*. However, fumigation in space 50% filled up with wheat showed 57.5% efficacy and in space 95% filled up with wheat mortality was 34% only. Our results are in a good agreement with the results of Shaaya *et al*. (1997), Lee *et al*. (2004), and Rozman *et al*. (2008). Rozman *et al*. (2008) believe that the probable cause may be in a considerable sorption of cineole to the surface of wheat grains and poor permeability of cineole vapors' into seed inter-space which largely decreased the fumigation effect. Very good results were gained in a fumigation of an empty space, while results of fumigation of space occupied with wheat (50% and 95%) did not prove to be successful and acceptable. The effect of grain on significant decreasing of the effectiveness of cineole and relatively high price of cineole and other natural EO make them too expensive to be adopted for wider use. The susceptibility of the eggs of stored product insects varied depending on the species and the plant essential oil. S. oryzae eggs were highly tolerant to *Eucalyptus blakelyi* Maiden as well as Melaleuca fulgens R.Br oils at 25±0.5 °C ($LD_{50}$ 100 ml/l, >7 days) (Lee *et al*., 2004), Essential oils or their components showing fumigant action generally have low mammalian toxicity. Liu and Ho (1999) opined that essential oils generally have strong odours and therefore might impart odour to treated commodities. 1,8- cineole, a common monoterpenoid in essential oils of plants, is used in expectorants and in cosmetic preparations (Rajaendran and Sriranjini, 2008). Future research should focus on residues on target commodity and the influence of any residues on product acceptability.

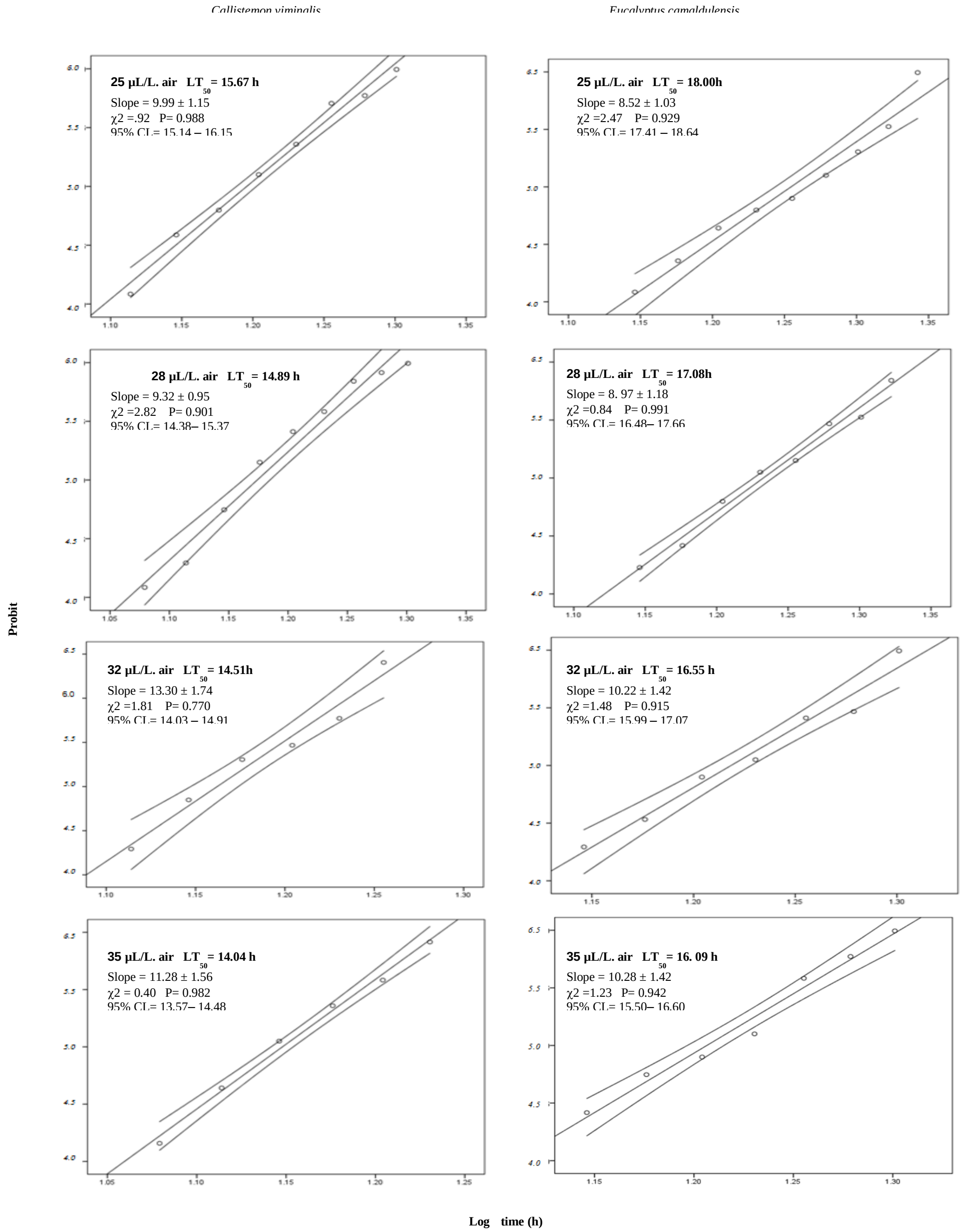
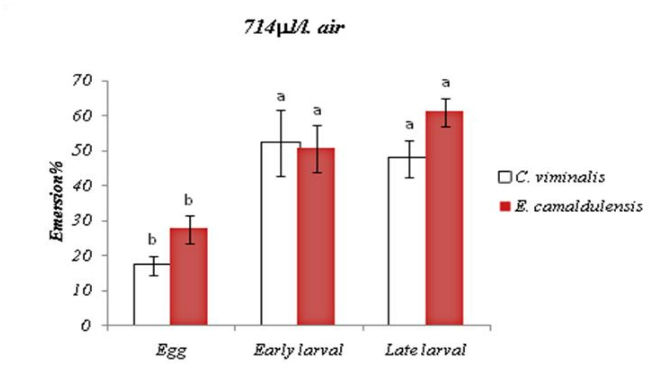



**Figure 1.** $LT_{50}$ values and time-response regression of different concentrations of Callistemon viminalis and Eucalyptus camaldulensis essential oils against Sitophilus granaries



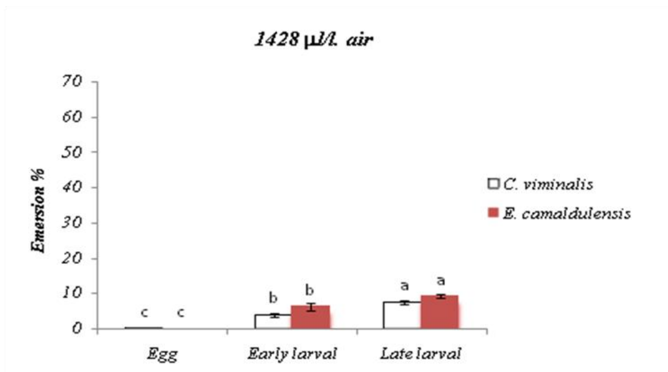




**Figure 2.** Effect of Eucalyptus camaldulensis and Calistemon viminalis essential oils on adults emersion (Mean ± SE) of Sitophilus granarius after treatment stages in jars with wheat grain Means followed by the same letter are not significantly different (Tukey, P<.05).

**Table 1**. $LC_{50}$ values and the relative toxicity of essential oils for controlling Sitophilus granarius, in empty jars

| Essential oil | n | p-value | $LC_{50}$ (%95 CL)[a] | Slope±SE | 2 (df)χ | RT (95% CL)[b] |
|---|---|---|---|---|---|---|
| C. viminalis | 350 | 0.605 | 9.73 (8.71-10.93) | 2.74±0.33 | 4.52 (6) | 0.19 (0.03-0.46) |
| E. camaldulensis | 350 | 0.171 | 18.21 (17.26-19.19) | 5.94±0.63 | 9.05 (6) | |

[a] $LC_{50}$ values are expressed as µL/L air of the essential oils with their 95% confidence limits.
[b] Relative toxicity = $LC_{50}$ value of C. viminalis to E. camaldulensis oil and 95% confidence limits.

**Table 2.** The relative toxicity for each essential oil tested in $LT_{50}$ for Sitophilus granarius

| Treatment | Relative toxicity | confidence limits95% |
|---|---|---|
| $A_{Ca}$: $A_{Eu}$ | 0.86 | **(0.81 - 0.91)*** |
| $B_{Ca}$: $B_{Eu}$ | 0.87 | **(0.81 – 0.92)*** |
| $C_{Ca}$: $C_{Eu}$ | 0.86 | **(0.80 – 0.91)*** |
| $D_{Ca}$: $D_{Eu}$ | 0.87 | **(0.79 – 0.92)*** |
| $A_{Eu}$ : $B_{Eu}$ | 1.05 | **(1.00 – 1.11)** |
| $B_{Eu}$: $C_{Eu}$ | 1.03 | **(0.98 – 1.08)** |
| $C_{Eu}$: $D_{Eu}$ | 1.02 | **(0.98 – 1.07)** |
| $A_{Eu}$: $C_{Eu}$ | 1.09 | **(1.03 – 1.15)*** |
| $B_{Eu}$: $D_{Eu}$ | 1.06 | **(1.01 – 1.12)*** |
| $A_{Ca}$: $B_{Ca}$ | 1.04 | **(1.00 – 1.10)** |
| $B_{Ca}$: $C_{Ca}$ | 1.04 | **(0.99 – 1.09)** |
| $C_{Ca}$: $D_{Ca}$ | 1.02 | **(0.98 – 1.07)** |
| $A_{Ca}$: $C_{Ca}$ | 1.09 | **( 1.04 – 1.15)*** |
| $B_{Ca}$: $D_{Ca}$ | 1.06 | **(1.01 – 1.12)*** |

**A: 25(µl/l air), B: 28(µl/l air), C: 32(µl/l air), D: 35(µl/l air), Eu: Eucalyptus, Ca: Callistemon**

**Table 3.** $LC_{50}$ values and the relative toxicity for each essential oil tested against *Sitophilus granarius*, in jars with wheat grain

| Essential oil | n | p-value | $LC_{50}$ (%95 CL)[a] | Slope±SE | 2 (df)χ | RT (95% CL)[b] |
|---|---|---|---|---|---|---|
| C. viminalis | 360 | 0.560 | 629.34 (520.12-769.92) | 1.67±0.21 | 2.99 (4) | 0.97 (0.75-1.24) |
| E. camaldulensis | 360 | 0.881 | 647.32 (547.75-760.04) | 2.01±0.24 | 1.18 (4) | |

[a] $LC_{50}$ values are expressed as µL/L air of pure essential oil with their confidence limits.
[b] Relative toxicity = $LC_{50}$ of C. viminalis to E. camaldulensis and confidence limits.

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