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# Chemical composition of *Cupressus lusitanica* and *Eucalyptus saligna* leaf essential oils and bioactivity against major insect pests of stored food grains

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ABSTRACT

The leaf essential oils from *Cupressus lusitanica*, Miller and *Eucalyptus saligna*, Smith obtained by hydrodistillation were analyzed by GC/MS and also screened for their insecticidal and repellent effects against adult *Tribolium castaneum, Acanthoscelides obtectus, Sitotroga cerealella* and *Sitophilus zeamais*. The *C. lusitanica* oil contained mainly umbellulone (18.38%) and α-pinene (9.97%) whereas the *E. saligna* oil was dominated by α-pinene (24.40%) and 1,8-cineole (24.26%). Bioassays showed that of the four insect species tested, *A. obtectus* and *S. cerealella* were the most susceptible to the oils, with $LC_{50}$ values of 0.05–0.11% v/w in contact toxicity and 4.07–7.02 μl/L air in space fumigation. Except in *T. castaneum* with percentage repellence (PR) values of 65–92.5%, the other test insects recorded PR values less than 30%. The PR values decreased with exposure time in all insects except in *T. castaneum*. Our results show that *C. lusitanica* and *E. saligna* essential oils are promising insecticides and repellents to be used against insect pests of stored food grains.



## 1. Introduction

Food insecurity in smallholder agricultural is largely due to crop pests, plant diseases, and poor storage and post-harvest handling techniques (Deng et al., 2009; Ogendo et al., 2012). In Africa a combination of arthropod pests and fungi often constitute the single greatest source of postharvest loss (≥50%) of stored products (Nukenine, 2010; Philips and Throne, 2010). Among insect pests, substantial post-harvest losses are caused mainly by *Sitophilus* spp., *Sitotroga cerealella* Olivier, *Tribolium castaneum* Herbst and *Prostephanus truncatus* Horn on cereals, *Acanthoscelides obtectus* Say and *Callosobruchus* spp. on grain legumes (Deng et al., 2009; Ayvaz et al., 2010). The maize weevil *Sitophilus* zeamais and Angoumois grain moth, *S. cerealella* are primary colonizers of maize both pre- and post-harvest exposing seed tissue to infestation by other insects, bacteria and fungi. Similarly, the red rust flour beetle, *T. castaneum*, is a secondary pest of stored cereal grains or other dried foods causing significant losses because it consumes grains. At elevated temperature and moisture conditions, damage caused by this beetle facilitates an accelerated growth of molds, including toxigenic species (Philips and Throne, 2010; Ogendo et al., 2012). Similarly, the bean weevil *A. obtectus* is one of the most destructive pests of the kidney bean, *Phaseolus vulgaris* L. with losses estimated at 30% in the Mediterranean region (Ayvaz et al., 2010).

In order to minimize cereal and legume losses, stored product insect pests are controlled using contact synthetic insecticides and fumigants including phosphine and methyl bromide, which is banned in some parts of the world. However, due to toxicity to humans and non-target organisms, insecticide resistance and resurgence of pests associated with synthetic insecticides, alternative remedies are being sought. Pesticides from natural sources, which are locally accessible and available, relatively inexpensive, biodegradable, less toxic to non-target organisms and less prone to resistance by insect species are considered potential candidates (Ogendo et al., 2012; Liang et al., 2013; Tucker et al., 2014). In this regard extracts from plants in the families Lamiacae, Ver-

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benaceae, Fabaceae, Leguminosae, Myrtaceae and Cupressaceae among others, have proven potent against a wide range of pre- and post-harvest insect pests (Koona, 2005; Karakoç et al., 2006; Polatoğlu et al., 2011; Athanassiou et al., 2013; Kariuki et al., 2013). Essential oils and their constituents are the most promising repellents (Mohan and Fields, 2002), contact toxicants (Rosman et al., 2007; Ogendo et al., 2008; Abay et al., 2012) and fumigants (Rajendran and Sriranjini, 2008; Campbell et al., 2010; Bett et al., 2013) against pests of stored products.

In previous studies, scientists have been prospecting Mexican cypress, *Cupressus lusitanica* (Cupressaceae: Pinales) and Sydney blue gum, *Eucalyptus saligna* (Myrtaceae: Myrtales) for protection of stored grains from insect infestation, pharmaceuticals and aromatherapy among other uses. The two aromatic plants are widely cultivated around the world as sources of fuelwood, electric poles, fencing posts, timber, ornamental purposes, shade and windbreaks. However, documented information indicate that *C. lusitanica* leaves are used to treat skin diseases caused by dermatophytes and to repel insects from stored grain and to alleviate coughs and cold symptoms (Kuiate et al., 2006). In addition, the essential oil is used in aromatherapy and massage to restore calmness, sooth anger, improve blood circulation, and treat coughs and bronchitis (Kamatenesi-Mugisha et al., 2013). In other studies, the essential oil has been reported to possess antibacterial activity against *Bacillus cereus* and antifungal activity against *Aspergillus niger* (Hassanzadeh et al., 2010). Similarly, *E. saligna* essential oil is also used as insect repellent and insecticidal agent (Brooker and Kleinig, 2006). In addition, the oil possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal and nematicidal (Batish and Kohli, 2008). The insecticidal activity of eucalyptus oils has been associated with components such as 1,8-cineole, citronellal, citronellol, citronellyl acetate, *p*-cymene, eucamalol, limonene, linalool, α-pinene, γ-terpinene, α-terpineol, alloocimene, and aromadendrene (Batish and Kohli, 2008). However, bioactivity and concentration of essential oils varies with species, season, location, climate, soil type, and age of the leaves, fertility regime, and methods used for drying the plant material and oil extraction (Brooker and Kleinig, 2006).

Considering the above prospects of essential oils as control agents of stored product insect pests, the current study purposed to; (1) determine chemical composition of *C. lusitanica* and *E. saligna* leaf essential oils, (2) evaluate contact and fumigant toxicity and repellence of the essential oils against *T. castaneum* (Coleoptera: Tenebrionidae), *A obtectus* (Coleoptera: Bruchidae), *S. cerealella* (Lepidoptera: Gelechiidae) and *S. zeamais* (Coleoptera: Curculionidae).

## 2. Materials and methods

### 2.1. Experimental conditions and rearing of test insects

Bioassays were conducted at the Integrated Biotechnology Laboratory, Egerton University at a temperature of 28 ± 2 °C and relative humidity of 65 ± 5% and 24 h darkness. Clean dry maize, wheat and bean grains, were placed in aluminum foil and kept in the oven at 100 °C for 24 h to eliminate any latent insect infestation. All the test insects were obtained from laboratory maintained cultures. *S. cerealella, S. zeamais* and *A. obtectus* were reared on whole maize, wheat and bean grains, respectively whereas adult *T. castaneum* were reared on broken wheat grains plus 5% brewer's yeast. In order to secure adults of the same age, all emerging adults were collected daily and transferred together in rearing jars for 2–5 days prior to use. Two to five days old emerging adult insects were used for bioassays.

### 2.2. Collection and preparations of plant materials

Fresh leaves of *C. lusitanica* and *E. saligna* were separately collected from branches of 7 year old trees from forestry demonstration plots in Busia, (0°27′20.02″N, 34°7′48.00″E, 1216 MASL), Kenya in August, 2012. On the spot identification of *C. lusitanica* and *E. saligna* species was carried out with the help of expertise, pictorial aids and literature materials (Kokwaro and Johns, 1998). Preserved specimens were forwarded to Prof. Samuel T. Kariuki, Plant Taxonomist, Department of Biological Sciences, Egerton University for authentic identification. The fresh leaf samples were air-dried under shade at ambient temperature (18–28 °C) for 14 days and further oven dried at 35 °C for 48 h. Dry leaf materials were then ground using an electric hammer mill (Wambua et al., 2011).

### 2.3. Hydro-distillation of essential oils, analysis and identification of essential oil constituents

The powdered material (500 g) of *C. lusitanica* and *E. saligna* leaves were hydro-distilled using a modified Clevenger-type apparatus for 4 h and the floating oil which separated from water, was collected. The oil was then dried over anhydrous sodium sulphate and stored in the refrigerator at 4 °C until use. Each test essential oil (1 μl) from the different plants was analyzed by gas chromatography (GC) coupled to mass spectrometry (MS) at the laboratories of the International Centre of Insect Ecology and Physiology (ICIPE), Nairobi on an HP-7890A (Agilent Technologies, Wilmington, USA) GC connected to an HP 5975C (Agilent, Wilmington, USA) mass spectrometer. The GC equipment was fitted with a non-polar HP-5MS capillary column 30 m × 0.25 mm internal diameter; 0.25 μm film thickness with 5%-phenyl methyl silicone as the stationary phase (J & W Scientific, Folsom, USA). The carrier gas was Helium (1.2 ml $min^{-1}$); oven temperature programmed at 35 °C (for 5 min) to 280 °C at 10 °C $min^{-1}$and then held isothermal at 280 °C for 10.5 min.; injection mode was splitless. Mass spectra were acquired at 70 eV within a mass range of 38–550 Daltons (Da) with a scan time of 0.73 scans $s^{-1}$ whereas the ion source was maintained at a temperature of 230 °C. Identification of the essential oil components was achieved on the basis of their retention indices (RI) (determined with reference to a homologous series of normal alkanes $C_5$–$C_{31}$) (Van Den Dool and Kratz, 1963).

The identity of essential oil constituents was further verified by comparison of their mass spectral fragmentation patterns with those reported in the mass spectra with library data (NIST05a and Adams MS HP, USA).

### 2.4. Instant contact toxicity bioassay

The instant toxicity of *C. lusitanica* and *E. saligna* leaf essential oils against adult *S. zeamais, S. cerealella, A. obtectus* and *T. castaneum* were conducted according to Asawalam et al. (2006) and Ogendo et al. (2008) with some modifications. Each test essential oil was applied to 10 g wheat and 20 g maize and bean grains in 100 ml glass jars at five concentrations (0.0, 0.05, 0.10, 0.15 and 0.20% v/w). The negative control consisted of untreated grains whereas Actelic Super (0.056% v/w), and crude soya oil (1.0% v/w) purchased from Meya Ltd., Nakuru served as positive controls. The grains were then artificially infested each with 20 unsexed adult test insects. The numbers of dead insects were recorded at 24, 72, 120 and 168 h post-treatment to estimate adult insect mortality. The percentage adult mortality was computed according to Asawalam et al. (2006)

and corrected for natural mortality using Abbott's formula (Abbott, 1925), respectively, in Eqs. (1) and (2)

$$\text{Actual Mortality}(\%) = \frac{N_D}{N_T} \times 100 \quad (1)$$

$$\text{Corrected Mortality}\,(P_T) = \frac{(P_O - P_C)}{(100 - P_C)} \times 100 \quad (2)$$

where $N_D$ and $N_T$ represent number of dead and total number of test insects per jar; $P_O$ represent observed and $P_C$ control percent mortalities.

### 2.5. Space fumigation bioassay

In fumigant toxicity *C. lusitanica* and *E. saligna* leaf essential oils were tested against adult stages of *S. cereallela, A. obtectus, S. zeamais* and *T. castaneum* in space fumigation chambers (Shaaya et al., 1991; Ogendo et al., 2008). Twenty unsexed adults ($N_T$) of each test insect species were introduced into meshed metallic cages with 5 g of food (grain) and suspended from a hook in a 3.4 L flat-bottom glass flask space fumigation chamber. Each test essential oil was separately applied to provide dosages of 0, 5, 10, 15 and 20 μl/L air on small pieces of Whatman No. 1 filter paper and then suspended in the chamber slightly below the cage. A magnetic stirrer was used to ensure even distribution of fumigant in the chamber over a 24 h exposure period in the experimental room maintained at temperature of 28 ± 2 °C and relative humidity of 65 ± 5%. The number of dead ($N_D$) insects was recorded 24, 72, 120 and 168 h post-fumigation. The percentage adult mortality was computed according to Asawalam et al. (2006) and corrected for natural mortality using Abbott's formula (Abbott, 1925) as in Eqs. (1) and (2), respectively as above.

### 2.6. Instant repellence bioassay

The repellence (choice bioassay) test was conducted according to Ogendo et al. (2008) and Liang et al., 2013 with modifications. The base of a 14-cm diameter plastic Petri dish was lined with aluminum foil, divided into four equal parts and 2.0 g whole/broken wheat or 4.0 g bean or maize grain samples placed in each quarter equidistant to the center. Each essential oil, dissolved in 1 mL acetone, was evaluated at five rates (0.00, 0.05, 0.10, 0.15 and 0.20% v/w) as an alternate untreated (control)-treated arrangement with four replicates per concentration. Control treatments consisted of choice bioassays with 0.5 μl/g of DEET (*N, N*-diethyl-*m*-toluamide) as positive control and crude soya oil (10.0 μl/g) and no-choice all untreated and as negative controls. The treated grains were kept for 1 h to allow the acetone to evaporate. Twenty unsexed adult stages of *S. cereallela, A. obtectus, S. zeamais* and *T. castaneum* were then released at the center of the Petri-dish and the top secured using a plastic cover. The number of insects present in the control ($N_C$) and treated ($N_T$) grains were recorded 1, 3, 5 and 24 h post-exposure. Percent repellence (PR) values were computed according to Ogendo et al. (2008).

$$\text{Percentrepellence}\,(\text{PR}) = \frac{(N_C - N_T)}{(N_C + N_T)} \times 100 \quad (3)$$

### 2.7. Statistical data analysis

Insect mortality data were corrected for natural mortality using Abbott's formula (Abbott, 1925). Data on percentage mortality and repellence were corrected for heterogeneity of treatment variances using arcsine-transformation (Leatemia and Isman, 2004) before being subjected to one-way ANOVA using JMP 9 software (SAS Institute, 2010). Means were separated by the Tukey–Kramer honestly significant difference (HSD) test at the 5% ($P < 0.05$) significance level (Sokal and Rohlf, 1995). The relationship between the oil concentration applied and percentage mortality was determined using Probit Regression Analysis of transformed (log base 10) data to estimate lethal concentration that kills 50% ($LC_{50}$) of test insects (SPSS, 2010). Any two $LC_{50}$ values in a column whose 95% confidence limits did not overlap were regarded as significantly different (Finney, 1971; Talukder and Howse, 1994).

## 3. Results

### 3.1. Chemical composition of C. lustanica and E. saligna leaf essential oils

The leaf essential oils obtained by hydrodistillation of both plants yielded 0.35 and 0.38% (v/w) of oil in *C. lusitanica* and *E. saligna*, respectively. Table 1 shows the retention time (min), retention index, chemical identity and relative percentage (%) concentration of chemical constituents. The GC–MS analyzes enabled the identification of a total of 68 compounds in *C. luistanica* oil corresponding to 99.98% of the total oil whereas in *E. saligna* 49 compounds were also identified accounting for 99.94% of the total oil composition (Figs. 1 and 2). The major constituents identified in *C. luistanica* oil were umbellulone (18.38%), α-pinene (9.97%), sabinene (8.16%) and limonene (7.91%). However, *E. saligna* oil was dominated by 1,8-cineole (24.26%), *o*-cymene (9.92%) and α-terpineol (8.81%). Comparing the chemical groups of the two oils, *C lusitanica* oil was dominated by oxygenated monoterpenes whereas that of *E. saligna* oil was mainly monoterpene hydrocarbons.

### 3.2. Instant contact toxicity

Instant toxicity bioassay showed that *C. lusitanica* and *E. saligna* leaf essential oils were toxic to adult *T. castaneum, A. obtectus, S. cerealella* and *S. zeamais*. The concentration of essential oil applied and time post-treatment significantly influenced the percentage adult mortality of all the test insects (ANOVA: $F_{(3,96)} = 6.9–293$; $^{***}P < 0.001$,). At 2.0% v/w, *C. lusitanica* oil caused 84.2%, and 86.0% mortality of *S. cerealella* and *A. obtectus,* respectively 24 h post-treatment (Fig. 3a). *T. castaneum* and *S. zeamais* was more tolerant with mortalities of 18.2 and 59.2% respectively 24 h post-treatment (Fig. 3a). Similarly, *E saligna* essential oil at 2.0% v/w, achieved 86.9% and 87.3% mortality in *A. obtectus* and *S. cerealella*, respectively, 24 h post-treatment (Fig. 3b). On the other hand, at the same concentration, the mortality in *S. zeamais* and *T. castaneum* were rather low, 10.0% and 11.8% respectively 24 h after treatment.

However, at longer exposure period moderate mortalities of 77.6% were observed with *C. lusitanica* oil against *T. castaneum* and 58% against *S. zeamais* 168 h post-treatment (Fig. 4a). Similarly, moderate mortalities of 56.3% were observed with *E. salgna* oil against *S. zeamais* and still low mortality of 19.7% in *T. castaneum* 168 h post-treatment (Fig. 4b).

*C. lusitanica* oil was highly toxic with $LC_{50}$ values of 0.05 and 0.11% v/w 24 h after contact for *S. cerealella* and *A. obtectus,* respectively. On the hand oil at the same concentration it was less toxic to *T. castaneum* and *S. zeamais* with $LC_{50}$ of 0.18 and 0.21% v/w respectively 24 h post-treatment. *E. saligna* oil had similarly high toxicity levels with $LC_{50}$ values of 0.02 and 0.08% v/w for *S. cerealella and A. obtectus* respectively 24 h post-treatment (Table 2). *T. castaneum* and *S. zeamais*, were more tolerant to *E. saligna* oil at the same concentration with $LC_{50}$ values of 0.19 and 17% v/w respectively 24 h post-treatments.

However, toxicity levels increased in *C. lusitanica* against *T. castaneum* and *S. zeamais* with $LC_{50}$ of 0.11 and 0.13% v/w respectively 168 h post-treatment (Table 2). *E . saligna* oil also became more

**Table 1**
Retention time (min), retention index and percent concentration (%) of chemical constituents of *Eucalyptus saligna* and *C. lusitanica* leaf essential oils.

| No[a] | Rt (min) | Compound name | RI[b] | % *E. saligna* | % *C. lusitanica* |
|---|---|---|---|---|---|
| 1 | 6.87 | 2,4-Dimethyl-3-pentanone | 804 | 0.09 | – |
| 2 | 8.35 | Isovaleric acid | 861 | 0.24 | – |
| 3 | 8.53 | 2-Methylbutanoic acid | 868 | 0.05 | – |
| 4 | 8.63 | (*Z*)-3-Hexenol | 873 | 0.02 | – |
| 5 | 8.82 | (*E*)-2-Hexen-1-ol | 880 | 0.02 | – |
| 6 | 9.24 | 1,2-Dimethyl-1,4-cyclohexadiene | 896 | 0.02 | – |
| 7 | 9.76 | 2-Methylpropyl-2-methylpropanoate | 918 | – | 0.12 |
| 8 | 9.85 | Tricyclene | 922 | – | 0.06 |
| 9 | 9.99 | α-Phellandrene | 928 | 0.03 | 0.99 |
| 10 | 10.12 | α-Pinene | 935 | 24.40 | 9.97 |
| 11 | 10.43 | α-Fenchene | 948 | 1.58 | 0.51 |
| 12 | 10.55 | Thuja-2,4(10)-diene | 954 | 0.11 | 0.06 |
| 13 | 10.72 | Benzaldehyde | 962 | 0.05 | 0.01 |
| 14 | 10.90 | 3-Methylbutyl propanoate | 969 | 0.12 | – |
| 15 | 10.95 | Sabinene | 972 | 0.31 | 8.16 |
| 16 | 11.29 | Myrcene | 987 | – | 2.29 |
| 17 | 11.34 | (*E*)-Dehydroxylinalool oxide | 989 | 0.14 | – |
| 18 | 11.58 | β-Phellandrene | 1000 | 0.16 | 0.48 |
| 19 | 11.66 | δ-3-Carene | 1005 | – | 6.93 |
| 20 | 11.72 | Isoamyl isobutyrate | 1009 | 0.14 | – |
| 21 | 11.80 | δ-2-Carene | 1013 | – | 0.53 |
| 22 | 11.95 | *o*-Cymene | 1023 | 9.92 | 5.81 |
| 23 | 12.02 | Limonene | 1027 | – | 7.91 |
| 24 | 12.10 | 1,8-Cineole | 1031 | 24.26 | – |
| 25 | 12.17 | (*Z*)-β-Ocimene | 1036 | 0.16 | 0.23 |
| 26 | 12.31 | Phenylactealdehyde | 1045 | 0.12 | – |
| 27 | 12.55 | γ-Terpinene | 1059 | 0.31 | 0.24 |
| 28 | 12.71 | (*E*)-Sabinene hydrate(IPP vs OH) | 1069 | – | 0.47 |
| 29 | 13.01 | *p*-Cymenene | 1092 | – | 1.33 |
| 30 | 13.25 | Linalool | 1101 | – | 3.91 |
| 31 | 13.32 | Isopentyl isovalerate | 1106 | 0.33 | – |
| 32 | 13.48 | *p*-1,3,8-Menthatriene | 1115 | – | 0.16 |
| 33 | 13.52 | endo-Fenchol | 1117 | 2.35 | – |
| 34 | 13.56 | α-Thujone | 1120 | – | 0.23 |
| 35 | 13.64 | *p*-(Z)-Menth-2-en-1-ol | 1124 | – | 0.61 |
| 36 | 13.72 | α-Campholenal | 1129 | 1.81 | – |
| 37 | 13.96 | [1S-(1α,3α,5α)]-6,6-dimethyl-2-methylenebicyclo[3.1.1]heptan-3-ol | 1143 | 7.13 | – |
| 38 | 14.03 | Camphor | 1147 | – | 0.62 |
| 39 | 14.11 | Camphene hydrate | 1152 | 0.53 | – |
| 40 | 14.24 | Sabina ketone | 1159 | – | 0.22 |
| 41 | 14.33 | Pinocarvone | 1165 | 3.02 | – |
| 42 | 14.39 | Borneol | 1168 | 4.57 | – |
| 43 | 14.51 | Umbellulone | 1175 | – | 18.38 |
| 44 | 14.54 | Terpinen-4-ol | 1177 | 1.52 | 6.12 |
| 45 | 14.66 | [α,α],4-Trimethyl-benzenemethanol | 1184 | – | 1.25 |
| 46 | 14.75 | α-Terpineol | 1189 | 8.81 | 1.98 |
| 47 | 14.86 | γ-Terpinen-7-al | 1207 | – | 0.19 |
| 48 | 15.06 | Verbenone | 1208 | 0.53 | – |
| 49 | 15.24 | Eucarvone | 1220 | – | 0.37 |
| 50 | 15.33 | Terpinolene | 1227 | 1.43 | – |
| 51 | 15.51 | Cumin aldehyde | 1238 | | 0.31 |
| 52 | 15.71 | Piperitone | 1252 | 0.28 | 1.19 |
| 53 | 16.19 | Thymol | 1284 | 0.27 | 0.76 |
| 54 | 16.30 | Benzyl isobutanoate | 1291 | 0.08 | – |
| 55 | 16.33 | Terpinolene | 1293 | – | 0.66 |
| 56 | 17.02 | α-Terpinene | 1342 | – | 2.60 |
| 57 | 17.10 | 2,2,5,5-Tetramethyl-3-cyclopenten-1-one | 1348 | 0.15 | – |
| 58 | 17.44 | α-Copaene | 1373 | 0.11 | – |
| 59 | 17.62 | Phenylethyl butyrate | 1386 | 0.19 | – |
| 60 | 17.71 | (*E*)-Jasmone | 1392 | 0.13 | – |
| 61 | 17.78 | 3-Isopropylbenzaldehyde | 1397 | – | 0.16 |
| 62 | 17.90 | Premnaspirodiene | 1407 | – | 0.09 |
| 63 | 17.98 | α-Cedrene | 1412 | – | 0.09 |
| 64 | 18.06 | (*E*)-Caryophyllene | 1418 | – | 0.18 |
| 65 | 18.31 | Germacrene B | 1438 | 0.08 | – |
| 66 | 18.37 | (*E*)-Muurola-3,5-diene | 1443 | – | 0.54 |
| 67 | 18.59 | α-Guaiene | 1459 | 0.15 | – |
| 68 | 18.61 | (*E*)-Muurola-4(14),5-diene | 1461 | – | 3.40 |
| 69 | 18.69 | α-Macrocarpene | 1467 | – | 0.19 |
| 70 | 18.76 | α-Curcumene | 1473 | – | 0.21 |
| 71 | 19.02 | Viridiflorene | 1492 | 0.09 | 0.00 |
| 72 | 19.01 | Epizonarene | 1492 | – | 0.73 |
| 73 | 19.07 | β-Macrocarpene | 1497 | – | 0.18 |
| 74 | 19.14 | β-Vetivenene | 1502 | – | 0.11 |
| 75 | 19.24 | Durohydroquinone | 1510 | 0.09 | – |
| 76 | 19.33 | (*Z*)-Calamenene | 1518 | – | 1.98 |
| 77 | 19.51 | α-Dehydro-ar-himachalene | 1533 | – | 0.35 |

Table 1 (*Continued*)

| No[a] | Rt (min) | Compound name | RI[b] | % *E. saligna* | % *C. lusitanica* |
|---|---|---|---|---|---|
| 78 | 19.59 | β-Calacorene | 1539 | – | 0.43 |
| 79 | 19.82 | γ-Gurjunene | 1558 | 0.05 | – |
| 80 | 19.83 | α-Calacorene | 1559 | – | 0.12 |
| 81 | 19.93 | Pogostol | 1567 | 0.08 | – |
| 82 | 20.03 | Spathulenol | 1576 | 0.43 | 0.05 |
| 83 | 20.11 | Caryophyllene oxide | 1582 | – | 0.23 |
| 84 | 20.11 | Globulol | 1582 | 0.17 | – |
| 85 | 20.43 | *iso*-Leptospermone | 1608 | 3.23 | – |
| 86 | 20.45 | 1,10-di-*epi*-Cubenol | 1611 | – | 0.35 |
| 87 | 20.51 | α-Colocalene | 1616 | – | 0.08 |
| 88 | 20.65 | β-Gurjunene | 1628 | 0.08 | – |
| 89 | 20.65 | β-Acoradiene | 1628 | – | 0.36 |
| 90 | 20.75 | (Z)-Cadina-1(6),4-diene | 1637 | – | 0.26 |
| 91 | 20.91 | β-Eudesmol | 1650 | – | 0.43 |
| 92 | 21.13 | Cadalene | 1670 | – | 0.14 |
| 93 | 21.33 | (Z)-14-nor-Muurol-5-en-4-one | 1688 | – | 1.89 |
| 94 | 21.46 | 10-nor-Calamenen-10-one | 1699 | – | 0.17 |
| 95 | 22.55 | (Z)-5-Hydroxy-calamenene | 1823 | – | 0.08 |
| 96 | 23.61 | Isopimara-9(11),15-diene | 1926 | – | 0.14 |
| 97 | 23.94 | Kaur-15-ene | 1961 | – | 0.03 |
| 98 | 24.26 | Sandaracopimara-8(14),15-diene | 1996 | – | 0.22 |
| 99 | 24.52 | 13-*epi*-Manool oxide | 2024 | – | 0.27 |
| 100 | 25.37 | Abietadiene | 2115 | – | 0.12 |
| 101 | 25.81 | Nezukol | 2163 | – | 0.68 |
| 102 | 27.35 | (*E*)-Totarol | 2342 | – | 0.08 |

– = Absent.
[a] No = Peak numbers as indicated in Figs. 1 and 2.
[b] RI=Retention index.

**Table 2**
$LC_{50}$ values (% v/w) of essential oils after 24–168 h of contact with four stored product insects.

| Plant EO/Insects[a] | *N* | Time (h) | | | |
|---|---|---|---|---|---|
| | | 24 | 72 | 120 | 168 |
| *C. lusitanica* | | | | | |
| *T. castaneum* | 20 | 0.18 (0.17,0.21)[b] | 0.17 (0.15,0.18) | 0.13 (0.12,0.29) | 0.12 (0.11,0.14) |
| *A. obtectus* | 20 | 0.11 (0.17,0.21) | 0.17 (0.15,0.18) | 0.13 (0.12,0.13) | 0.12 (0.11,0.14) |
| *S. cerealella* | 20 | 0.05 (0.03,0.06) | 0.02 (0.01,0.04) | 0.02 (0.01,0.04) | 0.02 (0.01,0.04) |
| *S. zeamais* | 20 | 1.21 (0.46,25) | 0.52 (0.29,4.01) | 0.19 (0.16,0.26) | 0.14 (0.12,0.17) |
| *E. saligna* | | | | | |
| *T. castaneum* | 20 | 0.19 (0.16,0.27) | 0.17 (0.13,0.25) | 0.15 (0.12,0.29) | 0.11 |
| *A. obtectus* | 20 | 0.02 | 0.001 | 0.001 | 0.001 |
| *S. cerealella* | 20 | 0.08 (0.01,0.15) | 0.06 | 0.04 | 0.02 (0.01,0.04) |
| *S. zeamais* | 20 | 17 | 0.39 (0.27,0.91) | 0.39 (0.23,3.1) | 0.13 (0.10,0.17) |

[a] Twenty unsexed adult insects in 4 replicates, were used for each concentration (% v/w).
[b] Figures in parentheses represent the lower and upper 95% confidence limits for the $LC_{50}$ values.

toxic to *S. zeamais* 168 h post treatment recording a $LC_{50}$ value of 0.13% v/w (Table 2). By comparison, all test insects were susceptible to the oils except *T. castaneum* . The positive controls, crude soya oil and Actelic super$^{TM}$ were toxic to test insects causing a mortality of 88.5 and 100% mortality, respectively 72 h post-contact with treated grains.

### 3.3. Space fumigation

Fumigant toxicity of *C. lusitanica* and *E. saligna* leaf essential oils against the four test insects resulted in significant essential oil concentration-, insect species- and fumigation duration-dependent insect mortality (ANOVA: $F_{(3,96)} = 5.8–197.0$; $^{***}P < 0.001$). At 10 μL/L air, *C. lusitanica* oil caused 90.6 and 100% mortality of adult *S. cerealella* and *A. obtectus*, respectively 24 h post-fumigation (Fig. 5a). The *E. saligna* essential oil, at 15 μl/L air, caused 94.7 and 100% kill for *A. obtectus* and S. *cerealella*, respectively, 24 h post-fumigation (Fig. 5b). *C. lusitanica* oil was relatively more toxic with 65.8 and 71.4% mortality in *S. zeamais* and *T. castaneum* 168 h post-fumigation with a higher concentration of 20 μl/L air (Fig. 6a). Similarly at a concentration of 20 μl/L air *E. saligna* oil caused mortality of 61.1 and 92.1% in *S. zeamais* and *T. castaneum*, respectively 168 h post-fumigation (Fig. 6b).

*C. lusitanica* oil was highly toxic with $LC_{50}$ values of 4.08 and 4.71 μL/L air against *A. obtectus* and *S. cerealella*, respectively 24 h post-fumigation. The *E. saligna* leaf essential oil was moderately toxic with $LC_{50}$ values of 6.71 and 7.02 and μL/L air for *S. cerealella* and *A. obtectus*, respectively 24 h post-fumigation. However, *C. lusitanica* at a concentration of 20 μl/L air was more toxic to *S. zeamais* and *T. castaneum* with $LC_{50}$ values of 13.54 and 15.28 μL/L air, respectively 168 h post-fumigation (Table. 3).

*T. castaneum* and *S. zeamais* were still less susceptible to *E. saligna* oil with $LC_{50}$ values of 9.49 and 15.34 μL/L air, respectively 168 h post-fumigation (Table. 3). The cumulative percentage mortality of all insects tested was higher 168 h post-fumigation compared to 24 h. *T. castaneum* was tolerant to plant oils as compared to the other insect species tested.

### 3.4. Instant repellence

The results of repellence assay for *C. lusitanica* and *E. saligna* leaf essential oil against *T. castaneum, A. obtectus, S. cerealella* and *S. zeamais* are presented in Fig. 7a and b. The plant species, concen-

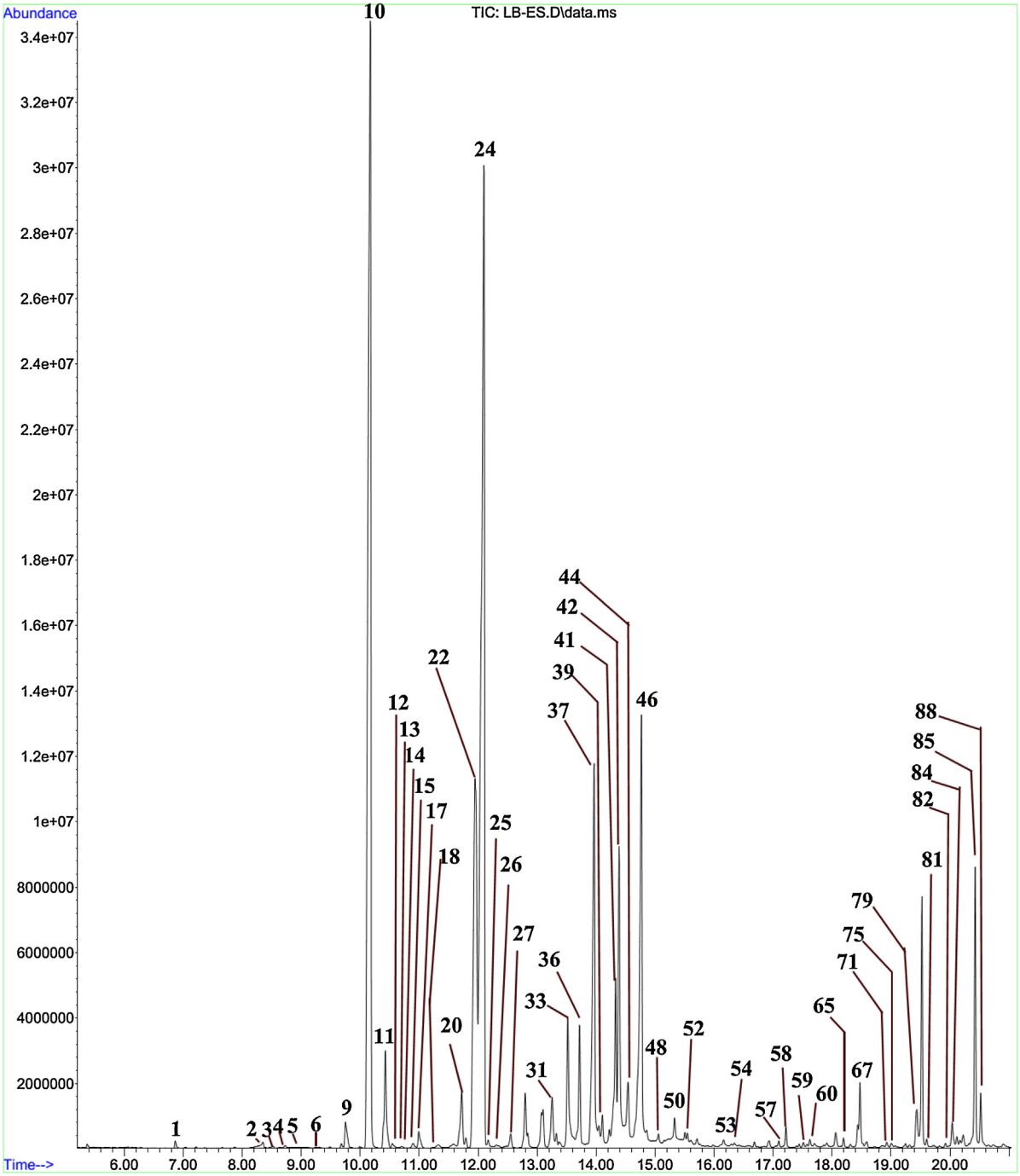


**Fig. 1.** Representative total ion chromatogram of the leaf essential oil of *Eucalyptus saligna*. Peaks 1–88 indicate the essential oil components identified (Table 1).

tration of essential applied and time post-treatment significantly influenced the percent repellence of all the test insects (ANOVA: $F_{(3,96)} = 2.37–63.83$; $^{***}P < 0.001$) except *A. obtectus* in which all factors were insignificant (ANOVA: $F_{(3,96)} = 0.431–2.42$; $P > 0.05$).

At 0.20% v/w, *C. lusitanica* leaf essential oil was strongly repellent to *T. castaneum* (92.5%) but produced low PR values against *A. obtectus* (27.5%) and *S. cerealella* (30.0%) 24 h after exposure (Fig. 7a). At the same concentration, *S. zeamais* showed negative (−5.3%) repellency (attraction) 24 h after exposure. The PR values for *E. saligna* leaf essential oil, at 0.20% v/w, against *T. castaneum, A. obtectus, S. cerealella* and *S. zeamais* were 9.3, 4.0, 1.8 and −10%, respectively 24 h after exposure (Fig. 7b). In *T. castaneum* the PR values increased (65–92.5%) with dosage 24 h post treatment with *C. lusitanica* oil. However, both *C. lusitanica* and *E. saligna* oils produced decreasing PR values of 12 to −4%, 55.5–1.8% and 38.9 to −10% against *A. obtectus, S. cerealella* and *S. zeamais*, respectively 24 h post-treatment (Fig. 7a and b). The positive control (DEET-treated grains) produced PR values of 2.5–30.5% after 24 h exposure, with low repellence observed against *S. zeamais* (30.5%) and *T. castaneum* (27.5%) as compared to *S. zeamais* and *S. cerealella* . The PR values for negative controls were zero and hence excluded from the results.

## 4. Discussion

The chemical profiles of *C. lusitanica* and *E. saligna* essential oils varied qualitatively and quantitatively in relation to the plant

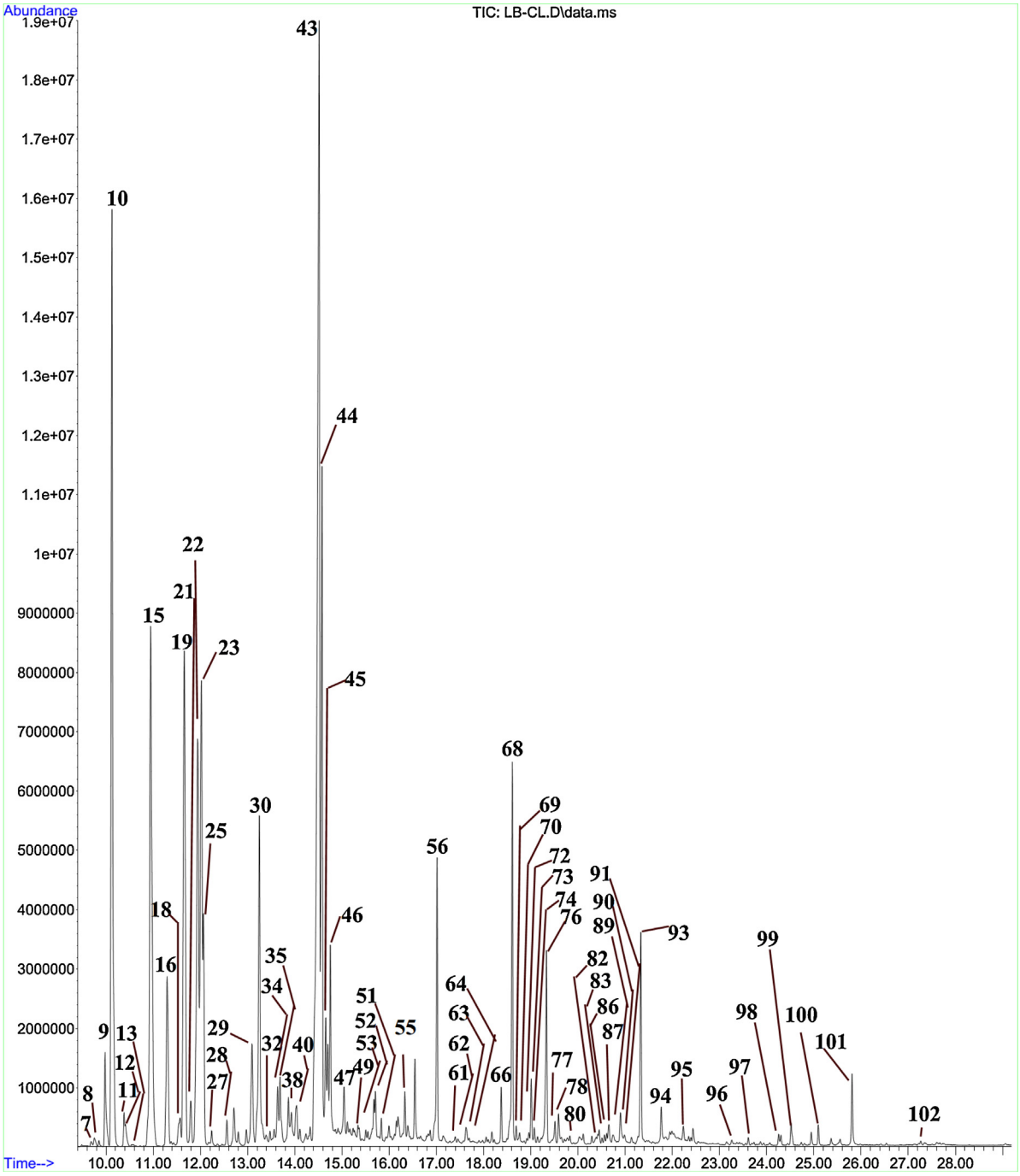


**Fig. 2.** Representative total ion chromatogram of the leaf essential oil of *Cupressus lusitanica*. Peaks 7–102 indicate the essential oil components identified (Table 1).

species, but were dominated by monoterpenoids; umbellulone, α-pinene and sabinene in *C. lusitanica* and α-pinene, 1,8-cineole, and *o*-cymene in *E. saligna* oil. Our results are in agreement with previous studies carried out on the essential oil of *C. lusitanica* found in Argentina (Floreani et al., 1982) and Portugal (Carmo and Frazão, 1989; Adams et al., 1997) containing mainly α-pinene (6.1–18.0%), β-pinene (13.0–16.5%), δ-3-carene (13–19.4%), abietadiene (11–24%), *trans*-totarol (5.1–6.5) and sabinene (6.7–13.0%). Whereas the essential oil of *C. lusitanica* collected from Monteverde, Costa Rica, was dominated by α-pinene (39.2–82.3%), limonene (4.2–17.6%), isobornyl acetate (4.6–9.6%) and *cis*-muurola-4,5-diene (6.4–6.7%) (Hassanzadeh et al., 2010) similar plants found in Cameroon are dominated by umbellulone (17–18%) and germacrene D (18.5%) (Kuiate et al., 2006; Teke et al., 2013). Noteworthy, whereas abietadiene and *trans*-totarol and germacrene D were not detected in the oils in the present study, they were detected in relatively high proportions in the oil obtained from C. *lusitanica* growing in Portugal (abietadiene (11–24%)) and *trans*-totarol (5.1–6.5%) (Adams et al., 1995) and germacrene D (18.5%) in the same plant species in Cameroon (Teke et al., 2013).

Similarly, results obtained for *E. saligna* revealed a chemical composition similar or different from those of other researchers. For instance, *E. saligna* growing in Cameroon contained mainly α-pinene (12.2–39.47%), cymol (12.7–41.1%), and 1,8-cineole (9.8–26.2%) (Tapondjou et al., 2005; Dongmo et al., 2008). Also, Mossi et al. (2011) reported that Cameroonian *E. saligna* was dom-

**Table 3**
$LC_{50}$ values (μl/L air) of essential oils against four stored product insects in space fumigation chambers 24 h post-fumigation.

| Plant EO/Insect[a] | *N* | Time (h) | | | |
|---|---|---|---|---|---|
| | | 24 | 72 | 120 | 168 |
| *C. lusitanica* | | | | | |
| *T. castaneum* | 20 | 19.67 (17.85,22.54)[b] | 19.02 (17.03,22.13) | 15.28 (13.81,17.24) | 15.28 (9.86,78.49 |
| *A. obtectus* | 20 | 4.08 (3.23,4.77) | 4.56 (3.71,4.98) | 3.61 (2.00,4.25) | 3.17 (0.83,3.99) |
| *S. cerealella* | 20 | 4.71 (4.01,5.27) | 3.69 (2.36,4.29) | 3.91 (2.88,4.45) | 3.76 (2.55,4.34) |
| *S. zeamais* | 20 | 29.11 (18.11,1139) | 20.84 | 17.11 (11.82,77.51) | 13.54 |
| *E. saligna* | | | | | |
| *T. castaneum* | 20 | 16.09 (11.96,30.47) | 11.47 (10.67,12.27) | 10.79 (8.12,13.30) | 9.49 (6.43,12.36) |
| *A. obtectus* | 20 | 7.018 | 5.37 | 5.09 | 5.06 |
| *S. cerealella* | 20 | 6.71 (6.25,7.48) | 5.03 (4.47,5.51) | 4.54 (3.65,4.87) | 6.71 (6.25,7.18) |
| *S. zeamais* | 20 | 26.85 | 30.79 (23.03,55.58) | 20.29 (16.78,28.13) | 15.34 |

[a] Twenty unsexed adult insects in 4 replicates, were used for each concentration (% v/w).
[b] Figures in parentheses represent the lower and upper 95% confidence limits for the $LC_{50}$ values.

inated by 1,8-cineole (45.2%), *p*-cymene (34.4%) and α-pinene (12.8%). However, *E. saligna* growing in Argentina contained a very high percentage of 1,8-cineole (93.2%) and other minor terpenes that include *p*-cymene, limonene, and α-terpinene (Toloza et al., 2006). These differences in the chemical composition of essential oils of *C. lusitanica* and *E. salgna* obtained from the present study and those analyzed in other regions could be attributed to differences in geographical and climatic factors associated with the regions these plants grow and possibly the method of extraction of these oils (Brooker and Kleinig, 2006). Consistent with these suggestions, Barton et al. (1989) observed that in *Eucalyptus* spp. the percentage of essential oils extracted and their chemical compositions varied widely between species and between individual plants.

Our results from the instant toxicity assay demonstrate that the essential oils obtained from the leaves of *C. lusitanica* and *E. saligna* are moderate to strong insect pest contact toxicants depending on the insect species, duration of exposure and concentration applied. The fact that *C. lusitanica* and *E. saligna* oils at a concentration of 2.0% v/w caused mortality in all test insects of 58–87% and 19–90%, respectively 168 h post-treatment is an indication of the promise the two pesticidal plants hold in pest management. These results are in agreement with other studies where several essential oils and constituents from plants in the Lamiaceae, Verbenaceae, Fabaceae, Cupressaceae and Myrtaceae families have demonstrated variable efficacy from weak to strong contact toxicants against major coleopteran and lepidopteran insect pests (Ngamo et al., 2004; Tapondjou et al., 2005; Ogendo et al., 2008; Mossi et al., 2011). In related studies, plant powders and essential oils from *Tephrosia vogelii* caused mortality of 83.0–93.7% in major lepidopteran and coleopteran pests of stored products (Ogendo et al., 2003, 2008). Interestingly, *C. lusitanica* oil was an effective insecticide against *T. castaneum* causing a mortality of 77.6% ($LC_{50}$: 0.13% v/w) 168 h post treatment.

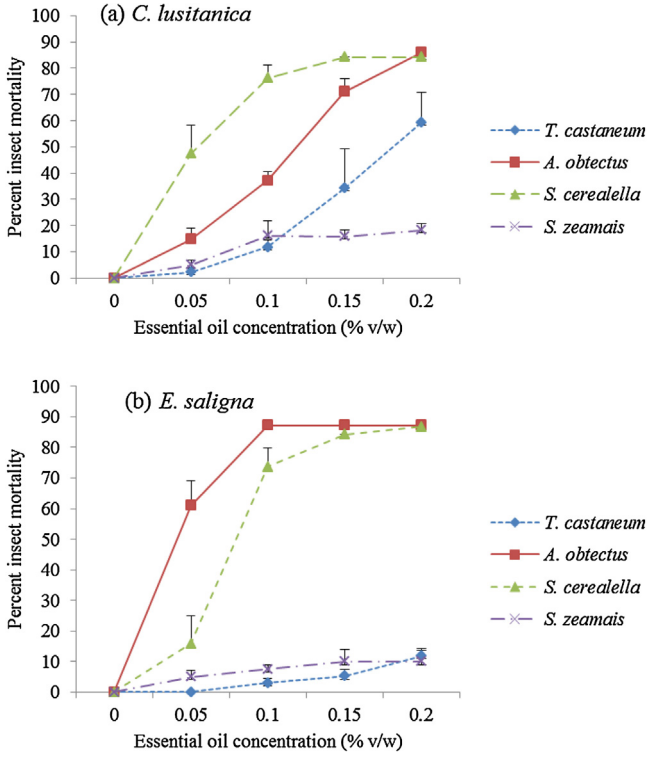


**Fig. 3.** Percent mortality (Mean ± SE, $n = 4$) of four stored product insects after 24 h of contact with (a) *C. lusitanica* and (b) *E. saligna* leaf essential oils.

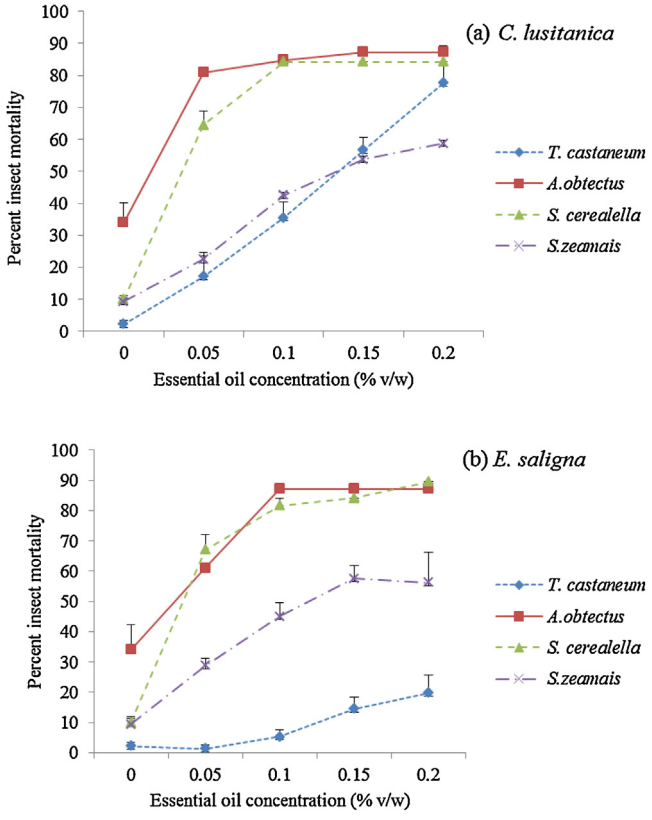


**Fig. 4.** Percent mortality (Mean ± SE, $n = 4$) of four stored product insects after 168 h of contact with (a) *C. lusitanica* and (b) *E. saligna* leaf essential oils.

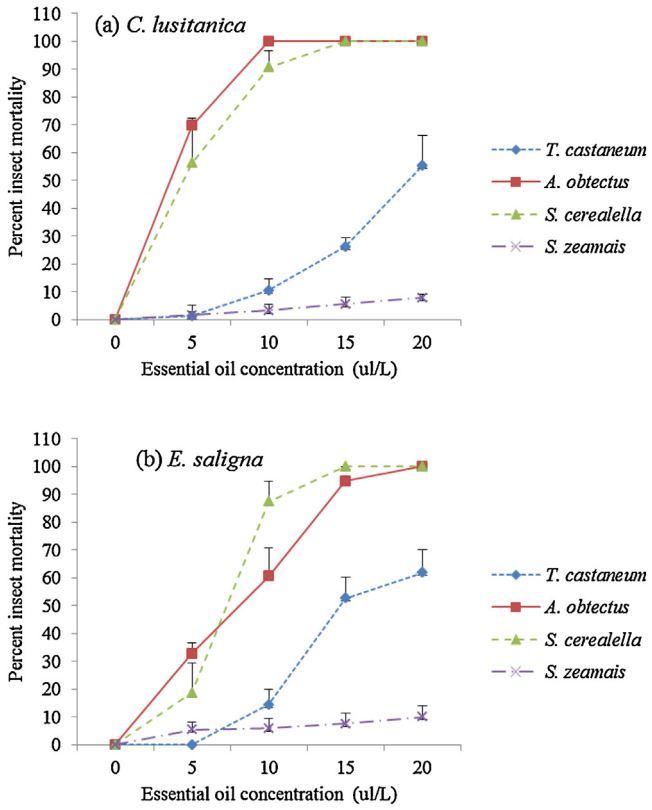


**Fig. 5.** Percent mortality (Mean ± SE, $n = 4$) of adult *T. castaneum*, *A. obtectus*, *S. cerealella* and *S. zeamais* after 24 h exposure to five concentrations (v/w) of (a) *C. lusitanica* and (b) *E. saligna* leaf essential oils in space fumigation chambers.

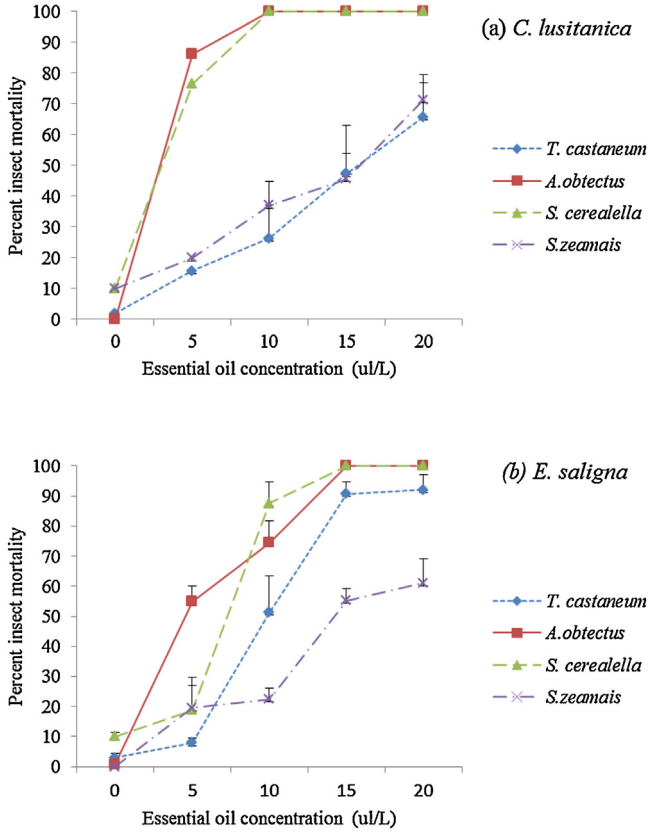


**Fig. 6.** Percent mortality (Mean ± SE, $n = 4$) of adult *T. castaneum*, *A. obtectus*, *S. cerealella* and *S. zeamais* after 168 h exposure to five concentrations (v/w) of (a) *C. lusitanica* and (b) *E. saligna* leaf essential oils in space fumigation chambers.

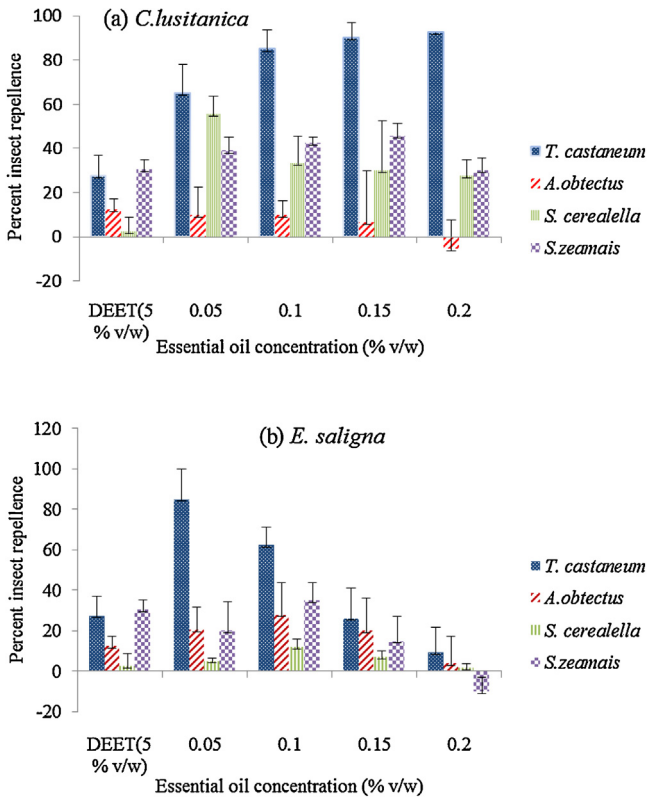


**Fig. 7.** Percent repellence (Mean ± SE, $n = 4$) of adult *T. castaneum*, *A. obtectus*, *S. cerealella* and *S. zeamais* after 24 h exposure to (a) *C. lusitanica* and (b) *E. saligna* leaf essential oils in untreated-treated choice bioassay system.

In previous studies, Ngamo et al. (2004) showed that essential oils of *Annona senegalensis* (Annonaceae), *Eucalyptus citriodora, Eucalyptus saligna* (Myrtaceae), *Lippia rugosa* (Verbenaceae) and *Ocimum gratissimum* (Lamiaceae) to had significant insecticidal activity against *S. zeamais*, but 50% of the efficacy was lost for all the plants except *A. senegalensis* 8 days post treatment. Tapondjou et al. (2005) demonstrated that oils from *E. saligna* and *Cupressus sempervirens* leaves assayed by impregnation on filter paper discs or coating onto maize grains showed that these chemicals caused significant mortality of *S. zeamais* and *Tribolium confusum. Eucalyptus* oil was more toxic than *Cupressus* oil to both insect species ($LD_{50} = 0.36$ mL cm$^{-2}$ for S. zeamais and 0.48 mL cm$^{-2}$ for *T. confusum*) and was more toxic to *S. zeamais* on maize ($LD_{50} = 38.05$ mL/40 g grain). It is possible that α-pinene (39.47%) and cymol (31.1%) which were major constituents in *E. saligna* essential oils were responsible for the higher toxicity.

Furthermore, Mossi et al. (2011) was able to demonstrate that essential oils of three *Eucalyptus* species caused mortality of 100% in *S. zeamais* at doses of 65, 100 and 400 μL for *Eucalyptus dunnii, E. saligna* and *Eucalyptus benthamii*, respectively. The $LD_{50}$ values of the three oils were 25. 03, 37. 93 and 121. 09 μL for *E. dunnii, E. saligna* and *E. benthamii*, respectively on filter papers impregnated with test essential oils. The authors were able to conclude that variations in toxicity of essential oils from the different species of *Eucalyptus* related to the concentration of 1,8-cineole and largely responsible for the oil toxicity (Duke, 2004).

The fact that plant oils were toxic at concentration of 4.08–7.02 μl/L air against *A. obtectus* and *S. cereallela* respectively 24 h post fumigation and 71.4–100% mortality in all test insects except *S. zeamais* 168 h post fumigation demonstrate their applicability in grain fumigation. The results of our study are mostly in agreement with the results of several previous investigators. Previous studies have demonstrated that *T. vogelii* essential oils pro-

duced strong contact (up to 83% kill) and fumigant toxicities against four coleopteran pests of stored cereal and legume grains including insects feeding on pigeon pea and chickpea (Minja et al., 2002; Papachristos and Stamopoulos, 2004; Ogendo et al., 2008). Lee et al. (2004) showed that essential oils from *Eucalyptus nicholii, Eucalyptus codonocarpa, Eucalyptus blakelyi, Callistemon sieberi, Melaleuca fulgens* and *Melaleuca armillaris* were effective against *Sitophilus oryzae* adults with $LD_{50}$ values of 19.0–30.6 mL/L air but were less toxic to *T. castaneum* and *Rhyzopertha dominica*. Toloza et al. (2006) showed that essential oils of *Myrcianthes cisplatensis*, *Eucalyptus cinerea, Eucalyptus viminalis* and *E. saligna* had knock-down time ($KT_{50}$) of 1.3–17.4 min against head lice, (*Pediculus humanus capitis*). Similarly essential oil constituents 1,8-cineole, anisole and benzyl alcohol had KT50 values of 12.0, 14.9, and 17.4 min, respectively against the same pest. In other studies, Rosman et al. (2007) found 1,8-cineole, camphor, linalool, thymol, borneol, extracted from *Lavandula angustifolia, Rosmarinus officinalis, Thymus vulgaris* and *Laurus nobilis* to cause up to 100% mortality in *S. oryzae* and *R. dominica* when applied for 24 h at the lowest dose (0.1 mL/720 mL vol.). However in *T. castaneum* no oil compounds achieved more than 20% mortality after exposure for 24 h, even with the highest dose (100 mL/720 mL volume). In related studies, Ogendo et al. (2008) reported that *Ocimum americanum* leaf essential oil at 50 $\mu L\,L^{-1}$ air, 7 days exposure and 120 h post fumigation time, caused 66, 95and 100% kill of *T. castaneum, S. oryzae* and *R. dominica*, respectively.

Alzogaray et al. (2011) found essential oils extracted from 11 species of the genus *Eucalyptus* and two of their hybrids to have fumigant activity against first instar *Blattella germanica* with lowest $KT_{50}$ values of 57.9–161.4 min by exposing nymphs to vapors emitted by 50 μL of essential oil in a closed container. The $KT_{50}$ values of monoterpenes from same oils were 38.8 for α-pinene, 55.3 for 1,8-cineole, 175.6 for *p*-cymene, and 178.3 min for γ-terpinene. The results of this study and pervious reports prove that plant oils have fumigant efficacy comparable to methyl bromide's recommended rate of 30–50 $g\,M^{-3}$ grain, 50 $\mu L\,L^{-1}$ air for the highly active Labiatae species oil, ZP51 and 50–150 $mg\,L^{-1}$ for allyl acetate to achieve 94.0–100% mortality of all insect pests of stored cereal and legume grains (Busvine, 1980; Shaaya et al., 1997; Rajendran and Muralidharan, 2005).

The reported moderate to strong contact and fumigant toxicity of the essential oils tested in the present study could be attributed to individual and/or blend effects of bioactive chemical constituents contained in the essential oils (Arriaga et al., 2005; Ogendo et al., 2008). Contact toxicity of essential oils against insect pests of stored products has been associated previously to presence of 1,8-cineole, eugenol, methyl eugenol, limonene and α-pinene among other bioactive essential oil constituents (Bekele and Hassanali, 2001; Huang et al., 2002). Moreover, essential oils constituents 1,8-cineole, camphor, linalool, thymol, borneol, limonene α-pinene *p*-cymene, and γ-terpinene have been associated with fumigant toxicity against various insect pests (Shaaya et al., 1991; Rosman et al., 2007; Alzogaray et al., 2011).

The results of contact and fumigant toxicity in the current study seems lower than those found by other researchers possibly due to differences in concentrations used and major chemical constituents of test plant essential oils. In the current study, the *C. lusitanica* oil contained mainly umbellulone (18.38%), α-pinene (9.97%), sabinene (8.16%) and limonene (7.91%) whereas the *E. saligna* oil was dominated by α-pinene (24.40%), 1,8-cineole (24.26%), *o*-cymene(9.92%) and α-terpineol (8.81%). The concentrations of 1,8-cineole was low in *E. saligna* and event absent in *C. lusitanica* which could have contributed to the variable efficacy as compared to other studies (Ngamo et al., 2004, Tapondjou et al., 2005; Mossi et al., 2011; Ogendo et al., 2013). These results, and those reported earlier, indicate that the insecticidal activity of the essential oils varies depending on the stage of the insect development, the species and the plant origin of the essential oil (Brooker and Kleinig, 2006).

Moreover, the insecticidal activity of eucalyptus oils has been associated with components such as 1,8-cineole, citronellal, citronellol, citronellyl acetate, *p*-cymene, eucamalol, limonene, linalool, α-pinene, γ-terpinene, α-terpineol, alloocimene, and aromadendrene (Duke, 2004; Batish and Kohli, 2008). However, it has also been suggested that individual substances contained in essential oils might have a mutual synergistic effect in evoking biological activity (Murungi et al., 2013). Lee et al. (2003) proved there was contact toxicity through the insect cuticle, and fumigant toxicity through the respiratory and digestive systems. Ryan and Byrne (1988) suggested that the toxic effect of essential oil constituents may be attributed to reversible competitive inhibition of acetylcholinesterase by occupation of the hydrophobic site of the enzyme's active center.

The results of repellency assay of essential oils of *C. lusitanica* and *E. saligna* showed variable responses from test insects. *C. lusitanica* essential oil was a strong repellent against *T. castaneum* at concentration of 0.20% v/w after 24 h of exposure and moderately repellent against *S. zeamais* but a weak repellent against the remaining insects. These results are in agreement with a previous study in which powders and essentials oils and constituents were strongly repellent against insect pests (Chebet et al., 2013). Chebet et al. (2013) demonstrated that grains treated with crude powders of *Tephrosia vogelii* and *Azadrachta indica* were equally the most repellent (PR values: 88–90%) against adult *Prostephantus truncatus* followed by *Lantana camara* (PR 73%).

In a related study, Toloza et al. (2006) showed that essential oils from *M. cisplatensis*, *E. cinerea, E. viminalis* and *E. saligna Mentha pulegium* and its benzyl alcohol component exhibited repellency indices of 75.5 and 57.8%, respectively against head lice, (*Pediculus humanus capitis*). Liang et al. (2013) screening for repellency against the *T. castaneum* and fourteen Chinese medicinal herbs showed that the essential oils from *Curcuma longa*, *Epimedium pubescens*, *Lindera aggregate*, *Nardostachys chinensis*, *Schizonepeta tenuifolia*, *Zanthoxylum schinifolium*, and *Zanthoxylum officinale* at doses of 8.5 $\mu lcm^{-2}$ after 2 h exhibited strong repellency against the pest(PR 37–94%). The repellence was associated with chemical constituents such as menthol, borneol, and eudesmol which showed repellency against the red flour beetles but weaker than DEET at lower concentrations.

The observed repellent activity could partly be attributed to the presence of monoterpenes and sesquiterpenes which are well-known repellents of phytophagous insects by acting in the vapour form on the olfactory receptors of these insects (Lee et al., 2003; Wang et al., 2006). The highly repellent effects of plant essential oil constituents such as α-pinene 1, 8-cineole, citronellol, eugenol, camphor, terpineol, limonene, geranial, neral, (*E*)-anethole have been demonstrated by other researchers (Tapondjou et al., 2005; Toloza et al., 2006; Mossi et al., 2011; Liu et al., 2011; Nivea et al., 2013). Similarly in the current study, *C. lusitanica* essential oil main constituents (umbellulone, α-pinene and sabinene) and *E. saligna* (α-pinene, 1,8-cineole, and *o*-cymene) could have contributed to the repellent activity of the two plants. However, minor essential constituents may contribute synergistically to the overall repellent activity of the major constituents (Mossi et al., 2011; Liu et al., 2011; Akhtar et al., 2012).

It is also evident that *C. lusitanica* and *E. saligna* essential oils are weak repellents against A. *obtectus*, *S. zeamais* and *S. cerealella* 24 h post-exposure. The results indicate also that repellence decreased with dosage and even negative repellence (attraction) observed. Similar results trend were observed by Wambua et al. (2011) who reported a dose- and exposure time-dependent negative repellence (attraction) of *Helicoverpa armigera* larvae to chickpea leaves treated with aqueous extracts of *T. vogelii*. Ogendo et al. (2003)

reported that maize grains admixed with Actellic Super$^{TM}$ 2% dust registered negative PR values against *S. zeamais* due to the arrestment of test insect by the chemical. In similar studies, Ogendo et al. (2008) reported eugenol to produced PR values in *C. chinensis* that decreased with dosage of extract. The major cause of the negative PR values was possibly due to the high contact toxicity of eugenol (Huang et al., 2002) against *C. chinensis*. *C. lusitanica* and *E. saligna* essential oils provide nothing significant as far as an effective repellent is concerned. However, negative repellence(attraction) could find a place in insect pest control especially in the push-pull strategy in integrated pest management where a protected source (crop) is unsuitable to pest (Push) while luring towards an attractive source (Pull) from where the pests are subsequently removed or killed avoiding residues in crop (Cook et al., 2007)

The results obtained from this study provide the scientific rationale for use of *C. lusitanica* and *E. saligna* essential oils and constituents as insecticides and repellents in the protection of stored product insect pests. The essential oils may be used as aromatized powders in contact toxicity. Since essential oils are already volatile substances they may be applied as tablets/encapsulated formulations (similar to done to phosphine) and aerosols in fumigation and repellence compared to methyl bromide and phosphine. Moreover, provided with a proper formulation and dosage, the plant essential oils may be exploited for use against insect infestation at the small scale farmer's level since they may be more effective and less cumbersome than application of dangerous synthetics. Therefore, if the problem of cost-effective commercial production and formulation can be solved, the essential oils tested could find a place in IPM strategies, especially where the emphasis is on environmental and food safety and on replacing the more dangerous synthetic repellents and insecticides.

## Conflict of interest

The authors of this manuscript are not in the know of any personal and institutional affiliations, financial support or membership that might be perceived as affecting the objectivity of this manuscript.

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