RepeLlents are a main tool to prevent the outbreak of mosquito-borne diseases that represents a threat for millions of people worldwide. Plant-based products are very promising, low-toxic and eco-friendly alternative to synthetic repellents. Here, we performed an olfactory screening of the essential oils (EOs) of Artemisia verlotiorum Lamotte (Asteraceae), Lavandula dentata L. (Lamiaceae), and Ruta chalepensis L. (Rutaceae) for their possible use as ingredients in topical repellents. The EOs smell profiles were then matched with their repellence against the mosquito Aedes albopictus (Skuse) (Diptera Culicidae). To obtain a more complete bioactivity description, we also tested the EOs oviposition deterrence and the larvicidal activity. The best smell profile was associated with A. verlotiorum EO, while R. chalepensis EO showed the lowest overall pleasantness. All the EOs had a significant activity as skin repellent against A. albopictus, deterred the oviposition in the field, and exerted a clear larvicidal activity. Beside the best smell profile, A. verlotiorum EO showed also the longest lasting repellent effect, assuring the complete protection of the treated skin against A. albopictus for a time 60% longer than the synthetic repellent DEET.

The Asian tiger mosquito, Aedes albopictus (Skuse) (Diptera Culicidae) is ranked among the most invasive mosquito species in the world1. Native to the tropical and subtropical areas of Southeast Asia, in recent time, Ae. albopictus has spread across many countries including Europe, North and South America, and Africa2. Besides its aggressive daytime biting behaviour, the medical importance of A. albopictus is due to its ability to transmit many human pathogens and parasites (e.g. yellow fever, dengue fever, West Nile, Japanese encephalitis, St. Louis encephalitis, chikungunya viruses, filarial nematodes)3. Since no vaccines or drugs are available for most of the pathogens carried by Ae. albopictus, the control of mosquitoes remains the essential tool for the prevention of the transmission of many of them3. To date, the control of adult mosquitoes commonly relies on the use of synthetic insecticides and repellents, mainly belonging to organophosphates and pyrethroids5, but treatments with such chemicals are expensive, show scarce efficacy and have a strong environmental impact6. Also N,N-Diethyl-meta-toluamide (DEET), a synthetic compound that is the most common active ingredient in insect repellents, has been associated to relevant human health risks7. For these reasons, alternative natural insecticides and repellents are now very appreciated by consumers. Essential oils (EOs) of aromatic plants are considered among the most promising alternative to synthetic chemicals8,9. EOs are generally recognized as environmental friendly, easily biodegradable, minimally toxic to mammals and have shown repellent10,11, toxicant12–14, oviposition deterrent, growth and/or reproduction inhibition15,16 activities against different mosquito species. In some cases, the activity of these compounds is higher, or it has longer duration than synthetic chemicals17,18.

Because of their properties, EOs have been proposed as active ingredients in mosquito-repellent topical products19 as well as in repellent and insecticidal mists sprayed by automatic outdoor systems20. However, despite their efficacy, low cost and low toxicity, EOs still do not have a widespread application due to some limitations such as their high volatility, composition variability and strong smell. In fact, the sensory impact of EOs has been reported as one of the most negative aspects of their use21. To that regard, to ensure market success as ingredient in EOs-based environmental and topical repellents, EOs should have a high efficacy against the target insect coupled with a pleasant smell for the consumers. To date, however, no specific investigations have been performed to evaluate their bioactivity against the target insects and their impact on human sensorial system.

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Lamiaceae, Asteraceae, and Rutaceae are among the main families investigated for their anti-mosquito properties. Given this, in the present research, the EOs, extracted from *Artemisia verlotiorum* Lamotte (Asteraceae), *Lavandula dentata* L. (Lamiaceae), and *Ruta chalepensis* L. (Rutaceae), were evaluated for their efficacy as repellents, larvicides, and oviposition deterrents against *Ae. albopictus* together with their impact on human sensorial system.

### Results

#### Essential oils smell characterization.

All the tested EOs were characterised by the same “Smell intensity” ($F_{(2,36)} = 0.723, p = 0.764$), while the “Smell persistency” as well as the “Overall pleasantness” resulted significantly different ($F_{(2,36)} = 4.069, p = 0.026$; $F_{(2,36)} = 11.941, p < 0.001$, respectively). In comparison with the *R. chalepensis* EO, *L. dentata* EOs showed a lower “smell persistency” associated with a higher “Overall pleasantness” (Fig. 1).

In this context, the best smell profile was associated with *A. verlotiorum* EO, closely followed by the *L. dentata* EO. *R. chalepensis* EO was characterized by the worst smell profile with the highest number of off-flavours indicated by panellists (Table 1). Most part of the judges (69%) associated the *R. chalepensis* EO to bad emotions represented by “Repulsion”, while *A. verlotiorum* and *L. dentata* EOs were associated with the main possible use in “Environmental protection” followed by “Body care” (Fig. 2).

#### Essential oils chemical analyses.

The compositions (compounds with relative abundances >1%) of the essential oils (EOs) are reported in Table 2. The complete composition of the EOs can be found as Supplementary Table S1.

A total of 25 compounds have been identified in the *L. dentata* EO, accounting for up to 99.60% of the total composition. Oxygenated monoterpenes dominated its composition, representing over 90% of the total. Of this chemical class of compounds, camphor and fenchone added up to over 75% of the EO, showing relative

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Figure 1. Essential oils sensorial description. *Artemisia verlotiorum*, *Lavandula dentata*, and *Ruta chalepensis* main essential oils (EOs) sensorial descriptors ranked by panel components on a 0-to-10 scale. Histograms represent the mean of the ranks; different letters indicate significant differences (Tukey’s b, $P \leq 0.05$) for each descriptor among EOs; bars represent the confidence interval.

| Odour class/EO         | *A. verlotiorum* | *L. dentata* | *R. chalepensis* |
|------------------------|-----------------|--------------|-----------------|
| Vegetative odours      | Mint            | Mint         | Rosemary        |
|                        | Sage            | Rosemary     | Sage            |
|                        | Chamomile       | Herbaceous   | Chamomile       |
|                        | Citronella      |              | Citronella      |
| Spicy                  | Balsam           |              | Balsam          |
|                        | Cocoa           |              | Cocoa           |
|                        | Sandalwood      |              | Sandalwood      |
| Other                  | Honey           |              | Honey           |
| Off-flavors            | Medicinal       | Smoky        | Smoky           |
|                        | Fresh paint     | Resin        | Resin           |
|                        | Wet leather     |              | Wet leather     |
|                        | Animal          |              | Animal          |

Table 1. Main odours that characterized the smell of *Artemisia verlotiorum*, *Lavandula dentata*, and *Ruta chalepensis* essential oils (EOs).
abundances of 52.05 and 26.19%, respectively. Exo-fenchol follows, with a relative concentration of 4.19%. Finally, among monoterpene hydrocarbons (relative abundance of 7.67%) β-pinene (2.69%) and limonene (1.93%) were the most relevant compounds.

The 96.38% of the EO of *A. verlotiorum* was composed by 39 compounds, belonging to the monoterpenes, sesquiterpenes, phenylpropanoids and other non-terpene derivatives chemical classes. As in *L. dentata* EO, but

Table 2. Chemical composition (%) of the *Artemisia verlotiorum* e *Lavandula dentata* essential oils used in the assays. *Chemical constituents \(\geq 1\%\); LRI, linear retention index on DB-5 column.

| Constituents | LRI  | *L. dentata* | *A. verlotiorum* | *R. chalepensis* |
|--------------|------|--------------|-----------------|-----------------|
| α-pinene     | 941  | 1.2          | 1.2             |                 |
| camphene     | 955  | 1.3          |                 |                 |
| β-pinene     | 981  | 2.7          |                 |                 |
| myrcene      | 993  | 1.9          |                 |                 |
| limonene     | 1032 | 1.9          | 2.2             |                 |
| 1,8-cineole  | 1035 | 7.1          |                 |                 |
| cis-sabinene hydrate | 1070 | 1.0          |                 |                 |
| cis-linalool oxide (furanoid) | 1076 | 1.8          |                 |                 |
| fenchone     | 1089 | 26.2         |                 |                 |
| 2-nonanone   | 1093 | 56.7         |                 |                 |
| linalool     | 1101 | 2.2          |                 |                 |
| 2-nonanol    | 1101 | 3.5          |                 |                 |
| 2,6-dimethyl phenol | 1108 | 5.2          |                 |                 |
| exo-fenchol  | 1118 | 4.2          |                 |                 |
| chrysanthenone | 1127 | 34.3         |                 |                 |
| geijerene    | 1143 | 1.7          |                 |                 |
| camphor      | 1145 | 52.1         |                 |                 |
| β-pinene oxide | 1158 | 2.2          |                 |                 |
| borneol      | 1167 | 1.4          |                 |                 |
| 2-decanone   | 1193 | 1.7          |                 |                 |
| 2-nonyl acetate | 1237 | 14.6         |                 |                 |
| perilla aldehyde | 1273 | 1.0          |                 |                 |
| 2-undecanone | 1294 | 12.7         |                 |                 |
| 2-undecanol  | 1308 | 1.3          |                 |                 |
| β-caryophyllene | 1420 | 12.6         |                 |                 |
| α-humulene   | 1456 | 1.3          |                 |                 |
| 2-undecyl acetate | 1475 | 1.4          |                 |                 |
| γ-muurolene  | 1477 | 9.9          |                 |                 |
| bicyclogermacrene | 1495 | 1.2          |                 |                 |
| caryophyllene oxide | 1581 | 4.0          |                 |                 |
| selin-11-en-4-β-ol | 1653 | 3.0          |                 |                 |

Figure 2. Suggested use of essential oils. Suggested use (Environmental or Body care) of the *Artemisia verlotiorum*, *Lavandula dentata*, and *Ruta chalepensis* essential oils as assessed by panel test. Values represent the percentage of the suggested use.
A completely different chemical composition was found for \textit{Ruta chalepensis} EO: 19 compounds represented 98.84% of the complete composition, of which 93.40% was composed of non-terpene derivatives. 2-nonenone and the closely related ester 2-nonyl acetate were the most abundant compounds in this EO composition, accounting for up to 34.27%, chrysanthenone was the most represented compound of this class and of the entire EO composition, followed by 1,8-cineole (12.62%), the second most abundant chemical class of this EO, monoterpene hydrocarbons showed an almost halved (4.17%) relative abundance compared to \textit{L. dentata} EO, with myrcene (1.89%) and \(\alpha\)-pinene (1.19%) as the most represented ones.

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**Essential oils oviposition deterrence.** The tested EOs showed a clear ability to deter \textit{Ae. albopictus} oviposition. After 14 days, the mean number of eggs laid on the control Masonite strips were 273.91 \(\pm\) 21.79 whereas, with the EOs, the number of eggs on the strips ranged from 31.50 \(\pm\) 5.68 to 259.50 \(\pm\) 41.01 (Fig. 3). The reduction of oviposition was significant for \textit{A. verlotiorum} and \textit{R. chalepensis} EOs (\(F_{1,6} = 10.248, p = 0.019; F_{1,6} = 118.418, p < 0.001,\) respectively) but not for \textit{L. dentata} EO (\(F_{1,6} = 0.162, p = 0.701.\) OAI values were 0.52 \(\pm\) 0.07, –0.08 \(\pm\) 0.00, and –0.69 \(\pm\) 0.02 for \textit{A. verlotiorum}, \textit{L. dentata} and \textit{R. chalepensis} EOs respectively, and confirmed the deterrence of \textit{A. verlotiorum} and \textit{R. chalepensis} EOs with no significant difference between the two EOs (Kruskal-Wallis pairwise comparison, \(p = 0.506).\)

**Essential oils larvicidal activity.** All the tested EOs showed a clear larvicidal activity against \textit{Ae. albopictus}. The median lethal concentration (LC\textsubscript{50}) of EOs, calculated by probit regression showed that \textit{R. chalepensis} EO resulted the most toxic one with LC\textsubscript{50} = 93.60 µL L\textsuperscript{-1} while, \textit{A. verlotiorum} and \textit{L. dentata} EOs LC\textsubscript{50} values were 324.00 and 602.80 µL L\textsuperscript{-1}, respectively (Table 3). The relative median potency (RMP) analysis of probits showed that \textit{R. chalepensis} EO was significantly more effective than \textit{A. verlotiorum} and \textit{L. dentata} EOs (\textit{R. chalepensis} vs \textit{A. verlotiorum}, RMP: 0.232, CI: 0.032–0.597; \textit{R. chalepensis} vs \textit{L. dentata}, RMP: 0.194, CI: 0.025–0.497), while the difference between \textit{A. verlotiorum} and \textit{L. dentata} EOs was not significant (\textit{A. verlotiorum} vs \textit{L. dentata}, RMP: 0.839, CI: 0.401–1.613).
of DEET was 10 minutes (Table 5). At the same dose (0.04 µL/cm²) against females of *Aedes albopictus*, was lower than the *Artemisia verlotiorum* EO, able to repel females of *Ae. albopictus*, as active ingredient in insect repellent products22. However, an increasing number of evidences suggest that such synthetic repellents may trigger undesirable hazardous interactions with biological systems, with a potential to generate harmful effects22,23. As an alternative, over the last 50 years, many plant species have been screened as potential sources of repellents and insecticides. Even if, for most plant-derived products, the supposed low environmental and mammalian toxicity have not been sufficiently investigated24,25, in some cases, such plant-derived repellents are utilized as active ingredient in commercial repellent. This is the case of the *p*-menthane-3,8-diol (PMD), extracted from the lemon eucalyptus, *Coriandrum citriodora* Hook (Mirtaceae)26. Nonetheless, for their use

| EO                  | Dosea | MCPT   | CI     | MRPT   | CI     |
|---------------------|-------|--------|--------|--------|--------|
| *A. verlotiorum*     | 0.01  | 2.33   | 2.13–2.53 | 31.00 | 11.68–50.32 |
|                     | 0.02  | 11.00  | 10.46–11.54 | 43.00 | 32.27–53.74 |
|                     | 0.04  | 16.34  | 13.33–19.35 | 51.00 | 42.70–59.30 |
|                     | 0.08  | 21.03  | 18.26–23.80 | 63.00 | 25.79–100.21 |
| *L. dentata*        | 0.01  | 1.50   | 0.19–2.81  | 22.00 | 19.85–24.15 |
|                     | 0.02  | 10.25  | 4.88–15.62 | 41.00 | 38.85–43.15 |
|                     | 0.04  | 11.33  | 8.56–14.10 | 36.00 | 26.20–45.80 |
|                     | 0.08  | 11.50  | 0.00–23.17 | 75.00 | 21.32–128.68 |
| *R. chalepensis*    | 0.01  | 1.25   | 1.07–1.43  | 40.00 | 29.22–50.78 |
|                     | 0.02  | 2.20   | 0.70–3.70  | 49.00 | 44.71–53.29 |
|                     | 0.04  | 10.22  | 0.00–28.75 | 45.00 | 0.90–89.10 |
|                     | 0.08  | 16.65  | 0.00–46.49 | 94.00 | 66.09–121.91 |
| DEET                | 0.04  | 10.00  | 3.99–16.00 | 70.00 | 62.46–77.54 |

Table 4. Repellence of the essential oils of *Artemisia verlotiorum*, *Lavandula dentata*, and *Ruta chalepensis* against females of *Aedes albopictus*. aDose of the extract that repels 50% of the exposed insect; bDose of the extract that repels 95% of the exposed insect. Data are expressed as µL cm⁻² of skin; CI, Confidence Interval; (df), degrees of freedom; P, Pearson Goodness-of-Fit Test.

| EO       | Dosea | MCPT   | CI  | MRPT   | CI  |
|----------|-------|--------|-----|--------|-----|
| *A. verlotiorum* | 0.0008 (0.0005–0.0013) | 0.0004 (0.0003–0.0006) | 0.0002 (0.0001–0.0003) |
| *L. dentata* | 0.0271 (0.0108–0.1402) | 0.0114 (0.0057–0.0310) | 0.0574 (0.0265–0.1670) |
| *R. chalepensis* | 1.061 ± 0.066 | 1.145 ± 0.054 | 0.679 ± 0.034 |
| Intercept  | 3.307 ± 0.245 | 3.872 ± 0.205 | 2.487 ± 0.111 |
| χ² (df)   | 8.25 (4) | 8.42 (4) | 6.88 (4) |
| P         | 0.083 | 0.077 | 0.226 |

Table 5. Median complete protection time (MCPT) and Median residual protection time (MRPT) of *Artemisia verlotiorum*, *Lavandula dentata*, and *Ruta chalepensis* essential oils against *Aedes albopictus* females. µL/cm² skin; CI, confidence interval. Data are expressed as min.

**Essential oils repellent activity.** The arm in cage tests showed that the dose of *R. chalepensis* EO, able to repel females of *Ae. albopictus*, was lower than the *A. verlotiorum* and *L. dentata* ones. The median effective dose (ED₅₀) of EOs, showed that *A. verlotiorum* EO was the least effective of the three EOs, with ED₅₀ value of about 0.8 µL cm⁻² of skin, while ED₅₀ of *L. dentata* and *R. chalepensis* were 0.4 and 0.2 µL cm⁻² of skin, respectively (Table 4). RMP analyses of probits showed that the differences between *R. chalepensis* and *A. verlotiorum* (RMP: 0.274, CI: 0.135–0.555), and between *L. dentata* and *A. verlotiorum* (RMP: 0.526, CI: 0.277–0.955) are significant. No statistically significant differences were observed between *R. chalepensis* and *L. dentata* (RMP: 0.520, CI: 0.273–1.038). On the contrary, the duration of the effect was longer for *A. verlotiorum* compared to *L. dentata* and *R. chalepensis* EOs, with a statistically significant difference (Breslow test: χ² = 5.142, P = 0.02; χ² = 13.588, P < 0.001, respectively). Consistently, the duration of the residual protective effect was longer for *A. verlotiorum* with respect to *L. dentata* and *R. chalepensis* EOs with differences, considering all the doses tested, statistically significant between *A. verlotiorum* and *R. chalepensis* EOs (Breslow test: χ² = 6.063, P = 0.014). At the intermediate dose of 0.04 µL cm⁻² skin, the median complete protection time (MCPT) of the EOs, ranged from 16 to 10 min, for *A. verlotiorum* and *R. chalepensis*, respectively, while, the median duration of the complete protective effect of DEET was 10 minutes (Table 5). At the same dose (0.04 µL cm⁻² skin), the median residual protection time (MRPT) were 51, 36, and 45 min for *A. verlotiorum*, *L. dentata*, and *R. chalepensis*, respectively, while the residual protection of DEET ended after 70 minutes (Table 5). The complete set of repellence data (mean percentage of repellence ± standard deviation) can be found as Supplementary Tables S2, S3 and S4.

**Discussion**

Personal protection by mosquito repellents is a key measure for the control of several life-threatening vector-borne diseases and synthetic compounds (i.e. DEET, DEPA, IR3535, and Picaridin) are commonly used as active ingredient in insect repellent products22. However, an increasing number of evidences suggest that such synthetic repellents may trigger undesirable hazardous interactions with biological systems, with a potential to generate harmful effects22,23.
as active ingredients in topical or environmental spray products, EOs should not only be persistent and effective against mosquitoes but they also should be sensorially well accepted by consumers. To the best of our knowledge, there are no studies coupling the bioactivity of the EOs against insects and their sensory impact on customers. In fact, the sensory impact of EOs has been reported as one of the most negative aspects of their use.

Our results showed that *A. verlotiorum*, *L. dentata*, and *R. chalepensis* EOs are toxic and repellent against *Ae. albopictus* but very different for pleasantness and evocative effects to the human olfactory system. The experimental results showed that EOs with different chemical composition have different smell properties that can influence the consumer acceptability.

The panel test identified *A. verlotiorum* EO as the most pleasant-smelling one. The significant relative abundance of chrysantheneone in its composition is probably due to the choice of its panelists’ preference: Lawrence described it as a slightly oily-floral aroma suitable for aromatic industry. Together with 1,8-cineole and β-caryophyllene, it may be responsible for the vegetal fresh odour described by the panel. Chrysantheneone, indeed, has been reported as a strong but very pleasant smell contributor in the EO of another species of the same genus (*A. herba-alba*) by Rekkab et al. This oxygenated monoterpene aroma contribution has also been described as a sweet, floral scent in the EOs extracted from two other species of the Compositae family: *Laggera tomentosa* Sch. Bip. ex Oliv. et Hiern (Asteraceae) and *Otanthus maritimus* (L.) Hoffmanns. & Link (Asteraceae). A relevant contribution to *A. verlotiorum* EO aroma is also that of β-caryophyllene, which is commonly referred to as a “green” flavour contribution: its woody-spicy, dry and clove-like aroma is responsible for the aroma of leafy green vegetables. In our tests, the *A. verlotiorum* EO pleasantness score was closely followed by *L. dentata* EO, characterized by “Medicinal and Fresh paint” off-flavours that could be explained by the presence of camphor and fenchone as main chemical components. These compounds can, indeed, be associated with an intense pharmaceutical and chemical odour. In the *L. dentata* EO, the well-known warm, minty and ethereal aroma of camphor is coupled with the fenchone contribution. This oxygenated monoterpene has a powerful balsamic, almost pine-like, and spicy notes. On the contrary, the unpleasantness of *R. chalepensis* EO may due to the presence of 2-nonenone, a non-terpene derivative which determines a characteristic rue odour. Its strong fatty smell, with a very low odour threshold, is one of the most important contributors to soft mould-ripened cheeses and Blue cheese aromas. The closely-related ester, 2-nonyl acetate, exhibits a “fruiter” but unpleasant nuance, involved in the smell of ripening banana. It is, therefore, not advisable to use it in topical formulations, as its odour is strong and unpleasant.

The chemical composition of the *R. chalepensis* EO, tested in this work, showed some differences respect to the EOs from the same plant species tested in previous works against mosquitoes. *R. chalepensis* EO from Turkey, tested against *Aedes aegypti* L. (Diptera Culicidae) for biting deterrence, repellence, and larvicidal activities, showed different percentages of major compounds compared to the Italian one. In our experiment, *R. chalepensis* EO contains higher amounts of 2-nonenone and 2-nonyl acetate (56.73 and 14.16%, respectively) and a lower relative percentage of 2-undecanone (12.74%), which, for the Turkish ones, was 27.9, 10.6, and 43.2%, respectively. Similarly, the EO extracted from Tunisian cultivated *R. chalepensis* contained a lower relative percentage of 2-nonenone (20.5%) and a higher percentage of 2-undecanone (39.3%) compared to the EO from wild plants utilized in this experiment. Such differences in the chemical composition of EOs from the same plant species are expected and probably due to the different geographical area, growth stage and period of harvesting of the plant. The evolution of different chemotypes of plant species may represent a form of adaptation to the environment and can be considered as an advantage aimed at avoiding the development of resistance by insects. Dris et al. found that the EO composition of Algerian *L. dentata*, that showed a good larvicidal activity against *Culex longiareolata* (Macquart) and *Culex pipiens* L. (Diptera Culicidae), was dominated by α-terpinolene, a monoterpane hydrocarbon not detected in the *L. dentata* EO of the present study, followed by a much lower relative percentage of camphor. In line with our findings, *A. verlotiorum* EO main compound, chrysantheneone, was also reported as a major compound of *Achillea millefolium* L. (Asteraceae). It exhibited significant toxicity rates against *Ae. albopictus* and *Cx. pipiens*, thus confirming the mosquitoes repellent effect exerted by this compound, even when present in another species.

In the present work, besides the chemical and sensorial characteristics, we described the repellent and toxic activities of *A. verlotiorum*, *L. dentata* and *R. chalepensis* EOs against *Ae. albopictus*. Results showed that *A. verlotiorum* and *R. chalepensis* EOs were able to significantly deter *Ae. albopictus* oviposition in open field with a reduction of the oviposition of 61.78 and 81.61%, respectively. In line with our results, other botanical compounds have been recognized as oviposition deterrents against mosquito species. Benelli et al. found that neem extracts were able to deter *Ae. albopictus* oviposition up to 88.59% over control. Similarly, *Artemisia annua* L. (Asteraceae) crude extract at 200 ppm exerted oviposition repellence rates against *Ae. aegypti*, *Anopheles snellenis* Wiedemann and *Culex quinquefasciatus* Say (Diptera Culicidae) of 43.38%, 73.85% and 41.57% respectively, and the ethanolic extract of *Cassia obtusifolia* L. (Leguminosae) leaves showed a strong repellence against the mosquito *Anopheles stephensi* Liston (Diptera Culicidae) at the dose of 100 mg L⁻¹ achieving 75.5% of effective oviposition reduction.

Beside the oviposition deterrence, *A. verlotiorum*, *L. dentata*, and *R. chalepensis* EOs showed larvicidal activity against *Ae. albopictus*, with *R. chalepensis* that was significantly more toxic than the other two EOs. The toxicity of *R. chalepensis* EO tested in this work was, however, lower than one of the EO extracted from Tunisian cultivated *R. chalepensis* plants (LC₅₀ = 33.18μL L⁻¹) but, overall, in the range of the LC₅₀ values (LC₅₀ range = 11–194μL L⁻¹) reported for 22 plant species EOs against *Ae. albopictus* larvae in previous works (reviewed by Pavela).

The dose-response repellence bioassays showed that all the EOs tested had a relevant activity against *Ae. albopictus* females. Among the three EOs tested, *R. chalepensis* EO was the most effective one, resulting about two times more effective than *L. dentata* EO and four time more effective than *A. verlotiorum* EO (see Table 4). In general, our results are in line with the repellence against *Ae. albopictus* reported for other aromatic plants EOs.
Conti et al.46,47, evaluating the EOs of Hyptis suaveolens L., Salvia dorisiana Standl., Salvia longifolia Nutt., and Salvia sclarea L. (Lamiaceae), found RD_{50} values ranging between 0.4 and 1.0 nL cm^{-2}. Similar efficacy against Ae. albopictus was also found for Coriandrum sativum (Aptiaceae) (RD_{50} = 0.2 nL/cm²) skin)48 and for Tunisian cultivated R. chalepensis EOs (RD_{50} = 0.2 nL/cm²) skin)46.

EOs high volatility usually limits the duration of their efficacy49. For this reason, beside the effective dose, it is important to determine the EOs protection time in order to assess their possible practical use as mosquito repellents. A. verlotiorum, L. dentata and R. chalepensis EOs, besides low effective doses, also showed a very good persistency of the repellent effect that, in terms of complete protection time and residual effect, was similar or longer than the DEET one. Experiments on the lasting of the EOs repellence against Ae. albopictus are scarce. However, in line with our results, Conti et al.47 found that S. dorisiana, S. longifolia, and S. sclarea EOs were able to completely repel Ae. albopictus for times ranging from 31 and 21 min at 0.04 µL cm^{-2}. Similarly, Das et al.50 showed that Curcuma longa L. (Zingiberaceae), Pogostemon heynceanus Benth. (Lamiaceae), and Zanthoxylum limonella Alston (Rutaceae) EOs were able to significantly repel laboratory reared Ae. albopictus mosquitoes up to 23 min at the dose of 5% (about 5 times more concentrate than the solutions tested in the present study). Moreover, Nasir et al.51, testing Mentha piperita L. Ocimum basilicum L. (Lamiaceae), and Zingiber officinal Rosc. (Zingiberaceae) EOs at 10% (about 10 times more concentrate than the solutions tested in the present study), obtained protection times ranging from 34 to 98 min.

It is noteworthy that the A. verlotiorum, L. dentata and R. chalepensis EOs effective repellent dose was not consistent with the lasting of their effect. A. verlotiorum EO, although the least effective after the skin treatment, resulted the longer-lasting one among the three tested EOs, assuring at 0.04 µL cm^{-2} skin a complete protection of the treated skin 60% longer than that of the synthetic chemical repellent DEET. On the contrary, R. chalepensis EO, although the most effective, resulted the less persistent one. Low persistence of R. chalepensis EO was previously observed also by Conti et al.46. Such differences in the duration of the effect may also be due to the addictive, synergistic or antagonistic interactions of EOs different compounds, with different volatility52.

In this study, A. verlotiorum, L. dentata and R. chalepensis EOs, showing anti-mosquito properties similar or higher than DEET, appear as viable alternative to synthetic repellents. Unlike DEET, EOs based products may be well accepted by the public due to the increasing consumer demand for effective and safe, natural products. More recently, considering the sensory quality parameters of EOs, the possibility of their use can be expanded by incorporating EOs in sprays, creams, and aerosols based anti-mosquito formulations. The good protection time currently observed can be further increased by polymer encapsulation53 and by other compound like vanillin54 or by the combined action of optimized EOs mixtures, due to the synergistic co-repellent effect of EOs chemical constituents55.

In conclusion, aromatic plants EOs are a valid alternative to synthetic repellents and insecticides for personal and environmental protection against mosquitoes. A new “integrated approach”, deriving from the merging of the repellence test, chemical, and sensorial data, can be useful to identify (or to select among those already existing) new and more appropriate EOs able to ensure the consumers’ compliance and an optimal protection level against mosquitoes.

Methods

Plant material, essential oil extraction, and chemical analyses. A. verlotiorum plants were collected along the river Arno in the Pisa area (Tuscany, Italy), R. chalepensis in the Northern Apennines (Monte Pisano) (Tuscany, Italy) and L. dentata in Wadi Thee Ghazal, near Taif (Makkah Province), Saudi Arabia. Plant species were selected among those with floral smell essential oils, the most suitable, in our opinion, to be proposed for the formulation of topical products. In addition, the selected plants are available in many countries with remarkable abundance so their future use in dermological products seems feasible. Plants were collected at the flowering stage in May 2016, air-dried in the shadow at room temperature, ground and then subjected to hydro-distillation for 2 h in a Clevenger-type apparatus. EOs were collected over water, dried over anhydrous sodium sulphate and stored at 5°C until analysis. The gas chromatography (GC) analyses were performed as described by Bedini et al.55. The identification of the constituents was based on the comparison of the retention time with those of authentic samples, comparing their linear indices relative to a series of n-hydrocarbons and on computer matching against commercial library of mass spectra56 as well as a homemade library of mass spectra built up from pure substances and components of known oils and mass spectra literature data.

Essential oils smell characterization. The smell profiles of the essential oils were evaluated by a trained panel composed by 13 assessors (“expert panel” of the Department of Agriculture, Food and Environment (DAFE) of University of Pisa). All assessors had previous experience in sensory descriptive analysis57,58. The assessors were provided with a specifically developed sensorial sheet consisting of a non-structured, parametric, descriptive scoring chart. The panellists described the main odours characterizing each sample on the basis of defined descriptors (see Tab. 7) ranked on a scale of 0–10 in terms of “Smell intensity”, “Smell persistency”, and “Overall pleasantness” and “Repulsion” as hedonic parameter59. Assessors were also asked to provide indications about the possible use (Environmental or Body care) of the EOs. The blind smell test was performed in the morning, in a well-ventilated quiet room and in a relaxed atmosphere. Each panellist was provided with a 2 x 2 cm filter paper soaked with 50 µL of an unknown EO. To avoid cross contamination, the three samples were assessed separately in the same morning (15 minutes waiting between the assessing).

Essential oils oviposition deterrence. The oviposition deterrence of the EOs was evaluated following the method described by Benelli et al.31. The experiments were carried out outdoor in an experimental field (about 3,000 m²) in Pisa (Italy) from June to September 2017. The EOs were tested at the concentration of 200
ppm in 0.1% Tween 80 tap water solution. For each treatment, four ovitraps filled with 500 mL of EOs solution were paired with four control pots containing Tween 80 0.1% solution only and each pair was separated by 5 m. A Masonite strip (200 × 25 mm) was placed in each pot. The Masonite strips were removed daily for fourteen days and the A. albopictus eggs were counted under a stereo microscope. The oviposition activity was expressed as oviposition activity index (OAI): OAI = (NT – NC)/(NT + NC), where NT is the total number of eggs in the test solution and NC is the total number of eggs in the control solution. Positive values (OAI > 0.3) indicate that the test solutions were attractive. Conversely, negative values (OAI < −0.3) indicate that the test solutions were deterrent.

Ae. albopictus rearing. Adults of Ae. albopictus originated from field-collected eggs. For eggs hatching, the Masonite strips were collected daily and placed in 250 mL beakers submerged in tap water under room conditions (26 ± 2 °C, 60% rh, photoperiod of 14:10 h (L:D). For larvicidal treatments, newly emerged larvae were fed with a small amount of cat food until fourth instar. For repellence tests, pupae were then placed in Plexiglas cylindrical cages (diameter 35 cm, length 60 cm) with a cotton stockinet access sleeve on the front. Emerged adults, were maintained (about 300 individuals per cage, sex ratio 1:1) under laboratory conditions, and supplied with 10% sucrose solution.

Essential oils repellent activity. The repellent effective dose (ED50) of the EOs was evaluated using the human-bait technique with some modifications. Experiments were conducted during the summer of 2017 in the above described cages, containing about of 150 field-derived, 8–12 days old females starved for 12 h that had not yet been either blood fed or exposed to any form of repellent. The study was approved by the ethical committee of the University of Pisa (Comitato Bioetico dell’Università di Pisa) and all experiments were performed in accordance with relevant guidelines and regulations. All volunteers provided written informed consent. Ten volunteers were chosen among subjects not allergic to mosquito bites. The volunteers had no contact with perfumed products on the day of the bioassay. After cleaning their hands in distilled water, their forearms were protected with a thick fabric sleeve and the hands with latex gloves, in which a dorsal square area 5 × 5 cm was cut open. The mosquito-exposed skin of one hand was treated with 100 μL of ethanol as a negative control. The other hand was treated with 100 μL of EO ethanolic solution at concentrations varying from 0.00001 to 0.8 μL cm−2. After about 5 min, the control hand was inserted inside the cage and exposed to mosquitoes for 3 min. Immediately after, the other hand was treated with the EO solution and, after about 5 min, exposed to mosquitoes in the same cage. The number of probing mosquitoes was recorded by two observers. On rare occasions, when no mosquito attempted to bite the untreated hand, the test was repeated with a new mosquito’s cage. The testing time was between 8:00 and 10:00 a.m. in order to perform the test during the peak of the biting activity of the species.

For the evaluation of the protection time, the tests were performed according to Nguyen et al. procedure, with few modifications. Four cages, as above described, were assigned to each participant, chosen as above described, and randomly used for the experiment. The randomized use of the cages was meant to reduce any bias associated with using only one cage and to prevent accumulation of repellent on or around the single cage, which may bias the evaluation of the effectiveness of the repellents. The experiment was performed as above described at the doses of 0.01, 0.02, 0.04, and 0.08 μL cm−2 skin. The procedure was repeated at an interval of 15 min. The lasting of the protection from mosquito bites (complete protection time, CPT), was calculated as the time between the application of the repellent and the first of two consecutive mosquito probing attempts. The lasting of the residual protection effect (residual protection time, RPT) was calculated as the time until no more differences in the number of probing were observed between the treated and the control hand. Data used to calculate the protection time were recorded by two observers. 100 μL of 1% N,N-Diethyl-3-methylbenzamide (DEET) (Sigma-Aldrich, Milan, Italy) ethanolic solution (0.04 μL cm−2 skin) was used as positive control, whereas 100 μL of ethanol was used as negative control.

Essential oils larvicidal activity. Groups of 20 fourth-instar larvae were placed in 250 mL beakers and exposed to 50, 100, 150, 200, 300, 400 and 600 μL L−1 of the EOs in 0.1% Tween 80 tap water solutions. As control, 20 larvae were exposed to the 0.1% Tween 80 tap water solution only. Mortality was recorded after 24 h. No food was given to the larvae during the tests. Three replicates for each treatment were performed. Mortality percentage rates were corrected using Abbott’s formula.

Statistical analysis. Data of the assessments of the smell profiles of EOs and oviposition were processed by one-way ANOVA with the EO treatment as factor. Equality of variances was checked before the analyses by Levene's test. Means were separated by Tukey’s post-hoc test. P < 0.05 was used as significance level of differences between means. OAI values were evaluated by Kruskal-Wallis test and means separated by pairwise comparison. The distribution of the time to mosquito-landing among EOs treatments and controls was estimated using the Kaplan–Meier method by repeated-measures modelling, with time as a categorical variable. Significant differences between MCPT and MRPT values were determined by Breslow (Generalized Wilcoxon) test. EOs larvicidal activity was evaluated by Log-probit regression. Since no mortality was registered in the control treatment, the larval mortality percentage rates were not corrected. Analysis were performed by the SPSS 22.0 software (IBM SPSS Statistics, Armonk, North Castle, New York, USA).

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Author Contributions
All named authors have contributed significantly to this work. S.B. and B.C. developed the study design. F.V., G.F., performed the sensory analyses. S.B. and A.B. extracted the plant essential oils and performed the chemical analyses. S.B., B.C. and J.G. performed the entomological part of the study. S.B. and F.V. performed the statistical analyses. S.B., F.V., R.A. and B.C., wrote the manuscript.

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