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Advances in mosquito repellents: effectiveness of citronellal derivatives in laboratory and field trials.

Short running title: *Advances in mosquito repellents: effectiveness of citronellal derivatives*

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ABSTRACT

BACKGROUND: Several essential oils, including that of Citronella (lemongrass, *Cymbopogon* sp., Poaceae), are well-known mosquito repellents. A drawback of such products is their limited protection time, due to the high volatility of their active components. In particular, the citronella oil protects for less than two hours, although formulations with fixatives can increase such time.

RESULTS: We synthesized hydroxylated cyclic acetals of citronellal, the main component of Citronella, to obtain derivatives with lower volatility and weaker odour. The crude mixture of isomers obtained in the reaction was tested under laboratory conditions for its repellency against two mosquito species, the major malaria vector *Anopheles gambiae* and the arbovirus vector *Aedes albopictus*, and found to be endowed with longer protection time with respect to DEET (N,N-diethyl-meta-toluamide) at the same concentration. Formulated products were tested in a latin square human field trial, in an area at a high density of *A. albopictus* for 8 h from the application. We found that the performance of

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the citronellal derivatives mixture is comparable (95% protection up to 3.5 hours) with those of the most widespread synthetic repellents DEET and Icaridin, tested at a fourfold higher doses.

CONCLUSIONS: Modifying hydrophilicity and volatility of natural repellents is a valuable strategy to design insect repellents with a long-lasting effect.

KEYWORDS: malaria, dengue, Chikungunya, essential oils, human bait, hydroxyl acetals

1 INTRODUCTION

The control of malaria, dengue, leishmaniasis and other vector-borne diseases can be efficiently obtained by reducing contact with the vector and consequent blood-feeding.[1] Topical repellents, house screening, insecticide-treated bed nets or dog collars are currently used with success to this purpose. While indoor protection can be obtained with different approaches, such as house screens, bed nets and insecticide spraying (mainly pyrethroids), topic repellents, to be applied on the skin or clothes, are basically the main personal protection method outdoor. There is a growing evidence that a substantial part of residual malaria transmission occurs outdoors thanks to behaviourally resistant mosquitoes that have previously been inside houses.[2] Moreover most of the vectors of dengue and Chikungunya are day time feeders, biting outdoors.

Therefore, topical repellents are particularly important to reduce outdoor transmission in areas where vector-borne diseases are endemic or epidemics.

Several plant extracts are well-known natural repellents against mosquitoes and other hematophagous arthropods.[3] Volatile compounds are detected through the insect's olfactory system and trigger an avoidance behaviour, being perceived as potentially toxic.[3,4] The main drawback of most plant-derived repellents is their short protection time, related to their relatively high volatility. An exception is menthane-3,8 diol (PMD), a compound obtained from the steam distillation of lemon eucalyptus (*Corymbia citriodora*).[3] The longer-lasting repellent effect of PMD is likely due to its lower vapor pressure, which in turn is related to the presence of two hydroxy groups in the molecule.

Citronellal is the main component in the essential oils of the citronella grass, *Cymbopogon nardus* and *Cymbopogon winterianus* (Poaceae).[5,6] These extracts, commonly indicated as citronella, are the most common natural compounds used as topic and spatial mosquito repellents in commercial products.[3] Formulations of topical repellents contain up to 5-10%, as higher concentrations can cause skin sensitivity.[3] Higher concentrations have been used in citronella-treated armbands[7] which are commercially available. The short protection time of the oil (about 2 hours) can be increased by microencapsulation or addition of components, such as vanilline, which do not have a repellent effect on their own.[3,4,8]

Several additives, such as liquid paraffin, salicylic acid, mustard and coconut oils, have been used in formulations of citronella-based repellents to increase the protection time.[9] Among them, vanillin is the most widely used additive and its effects has been tested with the main dengue vectors, *A. aegypti* and *A. albopictus*.[8,10-12] In any case, due to the limited duration of repellency, the U. S. Environmental Protection Agency requires that labels of citronella-based insect repellents report the recommendation to repeat applications every hour.[13]

The crude citronella oil has been mostly tested against health threatening hematophagous dipterans[4,7,14], but also against other blood-feeding arthropods such as triatomine bugs *Trypanosoma cruzi*[15,16], ticks[17] as well as insects attacking stored foods[18].

To the best of our knowledge, only few studies have used pure citronellal in such applications. The repellency effect against *Ae. aegypti* of 1 ml of pure citronellal applied to the forearm lasted less than 1 hour[4], while in a study testing wristbands impregnated with a 30% solution of citronellal report a repellency of 78% against the vector of lymphatic filariasis and epidemic encephalitis, *Culex pipiens pallens,* but did not report its duration.[7] Regarding toxicity, citronellal was found to interfere with cytochrome P450-mediated oxidation.[19] Moreover, cytochrome P450 and glutathione S-transferase were mostly inhibited by citronellal, as well as by other monoterpenes present in essential oils, when supplemented to fourth instar *Ae. aegypti* larvae together with piperonyl butoxide, a synergist component of pesticide formulations.[19]

As most of the natural repellents are efficient at high concentrations, a strategy to extend their protection time involves the use of derivatives with reduced volatility as reported by Iovinella and coworkers.[20] In this work, three cyclic ketals of menthone, which present reduced volatility with respect to the parent compound, were found to be active for longer times with respect to DEET against mosquito bites. Another advantage of these derivatives, again due to their low volatility, was a much weaker odour with respect both the parent menthone and to other products used as mosquito repellents. On the other hand, different derivatives of menthone, including its glyceryl acetal, have been reported to be endowed with insecticidal activity against different species of mosquitoes, such as *Culex quinquefasciatus*, *A. aegypti* and *Anopheles tessellatus*.[21]

With regards to citronellal derivatives, a patent has reported citronellal acetals against slugs, millipedes, and earthworms.[22] More recently, cyclic acetals obtained by reaction of citronellal with ethylene glycol and glycerol have been reported as mosquito repellents.[23] Such derivatives act also as repellents against the pharaoh ant (*Monomorium pharonis*), the best being those obtained with ethylene glycol and propanediol.[24]

With the aim to produce mosquito repellents with longer protection times, we have synthesised cyclic acetals by reaction of citronellal with glycerol, and tested their repellent activity against mosquitoes using the human bait method, along with the World Health Organization guidelines[25]. The mixture obtained from the synthesis, contained both hydroxy dioxanes and hydroxymethyl dioxolanes, and was tested against *A. albopictus* and *A. gambiae*. Our product was more efficient than DEET and Icaridin, and its protection time was further increased by the addition of vanillin, as well as when used in formulations.

2 MATERIALS AND METHODS

2.1 Synthetis methods

All reagents and solvents were from Sigma-Aldrich. DEET, Icaridin and Lagoon Protection® were supplied by Istituto Biochimico s.r.l. A 1 L, round bottom flask was charged with p-toluenesulfonic acid (1.0 g) and methanol (200 mL). While cooling in a ice bath, *rac*-citronellal (154 g, 180 mL, 1.0 mol) was added portionwise (approx. 10 mL per portion) to the magnetically stirred solution of the acid, so as to maintain the internal temperature in the flask below 10 °C. Trimethyl orthoformate (106 g, 109 mL, 1.0 mol) was added to the resulting clear solution and the cooling bath was removed. After the mixture had warmed to r.t., glycerol (100 mL, 126 g, 1.35 mol) was added in one portion and the mixture was concentrated under reduced pressure (approx. 8 mbar) while heating externally at 60 °C with a water bath. Sodium methoxide (5 g) was added to the residue in the flask and heating was prolonged for 15 min. After cooling to r.t., the mixture was partitioned between water (50 mL) and *n*-hexane (300 mL). The organic layer was dried over anhydrous sodium carbonate and then evaporated under reduced pressure to give the mixture of acetal products as a pale-yellow, clear oil (220 g, 96.5% yield).

2.2 Chemical analysis

The reaction products were analyzed by gas chromatography coupled to mass spectrometry on a GC-MS 7820 GC system-5977B MSD (single quadrupole, Agilent Technologies), by injecting 1 µl of a 200 ng/µl solution. The separation was carried out using a 19091S-433UI column (stationary phase, 95% PDMS, 5% benzene; 30 m × 0.25 mm, Agilent Technologies), using helium as carrier gas (1 mL min^{-1}). The oven temperature was programmed as follows: 45 °C (2 min); 10 °C min^{-1} up to 200 °C (3 min); 15 °C min^{-1} up to 300 °C (2 min). The injector port was set at 250 °C. Electronic ionization was carried out at 70 V and acquired m/z ranged from 50 to 550. Data were analyzed using Agilent MassHunter Qualitative Analysis B.07.00 software and spectra were checked for diagnostic ions expected based on the product structures. The NMR spectra were recorded in $CDCl_3$ at room temperature with a Bruker Avance DRX 400 spectrometer (401.36 MHz for ^1H and 100.93 MHz for ^{13}C). The proton data are summarized as follows: *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), *quint* (quintet), *dd* (doublet of doublets), *dt* (doublet of triplets), *td* (triplet of doublets), *m* (multiplet), *br* (broad signal). For referencing the chemical shift scale (δ), the resonances of the not deuterated residual solvent (^{1}H) or the deuterated solvent (^{13}C) were set to the recommended values.[26]

2.3 Estimastion of effective dose

The repellence of DEET, Icaridin and citronellal derivatives was evaluated using the human-bait technique (to simulate the condition of human skin on which repellents will be applied).[27-30] *A. albopictus* were reared and tested at 26 ± 2 °C, ≥60 ± 10 % relative humidity, and a 14:10 L:D photoperiod, within Plexiglas cylindrical laboratory cages (diameter, 35 cm; length, 60 cm) with one end closed by a net. During the tests, cages contained about 150 nulliparous, 4-7 day old, nonblood-fed females. Informed consent was obtained from four volunteers before they took part in this study. On the day of the bioassay, volunteers had no contact with lotions, perfumes, oils, or perfumed soaps. They wore latex surgical gloves, in which a dorsal square area of 30 cm^2 was cut open. Mosquito-exposed skin of one hand was treated with 100 µl of ethanol, as negative control. The other hand was treated with 100 µl of the reaction product in ethanol solution at increasing concentrations (0.005%; 0.02%; 0.05%; 0.10%; 0.50%; 1.00% corresponding respectively to 0.17; 0.67; 1.67; 3.33; 16.67 and 33.3 µg/cm^2). The control hand was exposed in the cage before the treated hand using the same test cage. The number of probing host seeking females in a 3-min exposure period was recorded. Before starting each replication, the mosquitoes' propensity to bite was assessed by inserting the control forearm into the cage and trials were continued only if at least 30 females performed probing behavior within 1 minute. Otherwise, the experiment was interrupted and resumed on a different day. The percentage of repellency obtained from all replicates (expressed as percentage protective efficacy, PE%) was calculated, according to the WHO/Guidelines for efficacy testing of mosquito repellents for human skin[25], at each dosage using the formula:

PE%=[(number probing untreated hand - number probing treated hand)/number probing untreated hand]x100

2.4 Estimation of protection time

To evaluate the protection time of the mixture of citronellal derivatives against *A. albopictus*, the PE% was measured, under the laboratory conditions described above, at intervals of 1 hour throughout 8 h. 100 μl of a 5 % ethanol solution, corresponding to 0.17 mg/cm^2 of exposed skin was applied on the test hand of 8 volunteers (4 females and 4 males). The control hand was treted with 100 μl of ethanol, as negative control. The protection time of DEET and Icaridin was measured at the same concentration (5%) and in the same conditions. To evaluate the role of vanillin as enhancer of protection time, the PE% against *A. albopictus* was measured, in the same conditions as above, by applying on the skin of four volunteers 100 μl of an ethanol solution containing 5 % of citronellal derivatives (0.17 mg/cm^2) and 1% of vanillin (34 μg/cm^2).

For tests against *A. gambiae* 100 adults 5-7 days post-emergence host-seeking females (reared, maintained and tested at 27 ± 2 °C, ≥80 ± 10% relative humidity, and a 12:12 L:D photoperiod) were placed in a laboratory metal cage with a net of Polyester mesh (W36 x D36 x H34); the PE% was measured with 1 mL of a 5 % solution of citronellal derivatives in ethanol applied on 600 cm^2 (corresponding to 83.33 μg/cm^2) of exposed forearm skin of 5 volunteers. For each volunteer, the test was performed every hour, up to 8 hours from the application. During each test, the control forearm was inserted in the cage for 30-seconds to verify that the number of landings and/or probings mosquitoes was ≥10 per 30 seconds. If less than 10 females attempted to bite the untreated hand in 30 seconds, the test trial was discarded and repeated with a new mosquito cage or postoned to the next day.

2.5 Field trials

The protection time of citronellal derivatives was measured in the field against *A. albopictus* at the concentration of 5% in ethanol. In these experiments, the PE% was measured by applying 1 ml of each formulated product on a surface area of 600 cm^2 (corresponding to 83.33 μg/cm^2) of the leg (knee to ankle). Protection time of Lagoon® (20% DEET, 0.5% geraniol, 79.5 % coformulants) and Icaridin (20% of active compound, PEG 400 20 %, denatured alcohol 29%, Parfum 0.5 %, in deionised water) were measured as a positive controls. As a negative control the same PEG formulation was used. A completely randomized design and blind test were adopted. Four volunteers (3 males and 1 female) and four collection sites were selected to match the number of compounds to be tested plus the control (PEG based formulation) as suggested by WHO guidelines[25]. The experiment lasted 7 hours and was repeated for 4 days. Each day volunteers were treated with a different compound at the beginning of the test. Each day each volunteers moved among the 4 collection sites that were separated by more than 50-100 meters from each other. Rotations were repeated at 15 minutes intervals and volunteers returned to the first site at one-hour intervals. The number of mosquitoes landing and/or probing on the skin was counted for 5-minutes at each site. The experiment was performed in the Botanical Garden of the University "Sapienza di Roma" (41°54′12.6″N and 12°30′59.7″E) between September and October 2021.

2.6 Statistical analysis.

Following Costantini and coworkers[31], we estimated the protection time of repellents by fitting a probit model under the assumption that the protection (p) of the treated skin from a mosquito could be expressed as $p = 1 - (T/C) = (C - T)/C$ where T and C are respectively the number of mosquitoes collected from the volunteer exposing the treated skin and that exposing the untreated skin. In the probit model, we considered the total number of mosquitoes collected every hour pooled over all replicates according to treatment and position. The complete protection time (CPT) for a given treatment was estimated from the time elapsed up to the first mosquito probing in each replicate. The median CPT and its confidence interval were estimated using the Kaplan-Meier survivor function procedure.

3 RESULTS AND DISCUSSION

3.1 Synthesis.

The acid-catalyzed condensation between citronellal and various glycols, performed under azeotropic removal of water[32], afforded samples contaminated with by-products from the acid-catalyzed cyclization of the unsaturated aldehyde. To prevent this problem, *rac*-citronellal was initially converted into the corresponding methyl acetal, followed by trans-acetalization of the latter with glycerol. After removal of the volatiles under reduced pressure, this two-steps procedure afforded the acetal in high yield and good GC-MS purity (96.5% and 94.3%, respectively), thereby allowing the use of the crude product without further purification. As expected[33], the ^{13}C NMR analysis of the sample revealed the presence of six distinct acetal compounds, whose C-2 carbon atoms were found to resonate in the δ_C = 101-105 ppm region. Based on previous spectroscopic studies on glycerol acetals of long-chain aldehydes[34,35], the NMR constants recorded for the sample were consistent with a mixture of *trans* and *cis* 1,3-dioxolane and 1,3-dioxane derivatives (Figure 1), whose relative content was roughly estimanted to be around 3.0:1.9:1.4:1.0 by least-square fitting of the intensities of selected ^{13}C NMR resonances (Supplementary Figure 1). In this regard it must be pointed out that for the six-membered ring derivatives, switching between the opposite configurations at the methyl-bearing stereogenic center in the side chain (C2') leads to enantiomeric structures, whose corresponding nuclei are isochronous and therefore undistinguishable under the achiral measument conditions adopted herein. On the contrary, because of the chirality of the 2,4-disubstituted-1,3-dioxolane the five-membered ring compounds that differ only in the configuration at C2' bear a diastereomeric (epimeric) relationship to each other. Under these conditions, the corresponding nuclei in each of the pairs of epimers are chemically non equivalent and may lead to distinct sets of NMR signals. This is what it is observed with the sample under exam, in particular in the region of oxygenated carbon atoms (Supplementary Figure 1) where, unlike the 1,3-dioxanes, all the resonances assigned to the *cis*- and *trans*-1,3-dioxolanes show up as pairs of narrowly spaced lines.

Not surprisingly[36], the GC-MS analyses of the reaction product showed only 4 peaks. These were tentatively assigned to the *cis* and *trans* diastereomers of the 1,3-dioxane and of the 1,3-dioxolane, respectively, on the assumption that -as much as for the enantiomers of the former- the silicone GC colum employed in our measurements could not separate to an appreciable degree the epimeric pairs of the latter. For all of them the molecular ion at m/z 228 was clearly visible. However, the mass spectra did not allow to assign the peaks to dioxanes or dioxolanes since in all the spectra the ion at $[M-31]^+$, expected for dioxolanes, was very faint.

3.2 Repellency in laboratory trials

The citronellal derivatives were tested for repellency against the tiger mosquito *A. albopictus* using the human-bait test, as described in the Materials and Methods. Figure 2 reports the average (±SE) of the protection efficacy obtained with four volunteers as a function of the dosage applied, as described in section 2.3, compared to those obtained for DEET and Icaridin. The compound reaches 95% repellency when used at the concentration of 0.1%, corresponding to 3.33 μg/cm^2 of skin. Raw data are reported in Table S1.

To evaluate the persistence on the skin of the mixture of citronellal derivatives, we measured its protection time against *A. albopictus* and *A. gambiae* (see section 2.4). For *A. albopictus* we used a dosage of 0.17 mg/cm^2 of exposed skin (30 cm^2) to 8 volunteers. DEET and Icaridin were also evaluated in the same conditions. Volunteers were asked to repeat the test at intervals of 1 hour throughout 8 hours. The results obtained are reported in Figure 3.

The 5% solution of crude citronellal derivatives shows a protection of 100% for 2 hours, and the repellency value is always more than 95% up to 8 hours (Figure 3A). Icaridin has a similar performance, displaying protection of 100% for 3 hours and a repellency always greater than 90%. Instead, DEET, used at a concentration four times lower than the one used in most commercial formulations (i.e. 5%), can protect only for 1 hour and its efficacy decreases very fast (after 2 h it drops to below 90%). The addition of 1% of vanillin to the 5 % solution of citronellal derivatives, resulted in an increase of the protection time. In fact, the mixture of citronellal derivatives shows protection of 100% for 5 hours and the repellency is higher than 98% for the following three hours, as reported in Figure 3A. This widely used additive with essential oils, commonly employed at higher concentrations, works as fixative by reducing the release rate of the volatile oil resulting in an improvement of protection time[3,8].

A similar experiment using then mixture of citronellal derivatives without additives has been conducted on *A. gambiae* (section 2.4) by applying the repellent solution on a larger skin surface (600 cm^2). As shown in Figure 3B, in this case the product can protect for at least 6 hours. Raw data are reported in Table S2.

The stability of citronellal derivatives, formulated and stored as reported in section 2.6, was very good with less than 0.01% of hydrolysed compounds.

3.3 Field trials.

In field experiments (Figure 4), protection from mosquitoes was estimated to be higher than 99% at the beginning for all tested compounds (Table 1): DEET (Lagoon: 20% DEET, 0.5% geraniol, 79.5 % coformulants), 100%; Icaridin (20% of active compound), 99.6% (95%CI: 98.9-99.9%) and the 5% mixture of citronellal derivatives 99.0% (95%CI: 98.2-99.5%). Repellency was still above 90% after 3.5 hours (DEET 99%, Icaridin 98.2% and citronellal derivatives 94.2%), and at 7 hours about 90% for Icaridin (93.8%) and about 80% for DEET and the mixture of citronellal derivatives (DEET 83.3% and the mixture of citronellal derivatives 79%).

Time from start of trial	**Mean protection (95% Confidence Intervals)**		
	Citronella derivatives (5%)	*DEET (20%)*	*Icaridin (20%)*
Start	99% (98.2 – 99.5%)	100% (99.9 – 100%)	99.6 (98.9 – 99.9%)
Half-time (3.5 hours)	94.2 (92.9 – 95.3%)	99% (98.4 – 99.5%)	98.2 (97.4 – 98.8%)
End (7 hours)	79% (76.1 – 81.6%)	83.3 (80.5 – 85.8%)	93.8 (92 – 95.3%)

Table 1. Results of probit model assessing protection (defined as one minus the ration between the number of mosquitoes landing on treated skin and those landing on the controls) measured during the randomized design and blind field trial test with 4 volunteers in a temperate area with high presence of *Aedes albopictus*. Estimates reported are predicted probabilities at three time points: Start = at the beginning of the trial, Half-time = at 3.5 hours from the beginning of the trial, End = at 7 hours from the beginning of the trial.

Despite a lower average protection (Figure 5) of the citronellal derivatives compared to DEET and Icaridin (Table 1), the rate of effectiveness reduction was comparable to Icaridin (see interaction term Time*Icaridin, in Supplementary Table S3 and Figure S2) and slightly better than DEET (see interaction term Time*DEET, in Supplementary Table S3) .

Of the three tested repellents, Icaridin was the best in terms of complete protection time (time to first landing/probing) with 128 minutes, followed by the citronella derivatives (88 minutes) and DEET (86 minutes). For reference, the first probing on untreated skin was observed in the first minute of exposure. The median complete protection time for the 5% solution of citronellal derivatives was 212 minutes (95% CI 120-320), *ie* probing was observed in half of the exposure event at this point, resulting lower than the other two repellents, tested at fourfold concentration (DEET: 324.5 (317-390), Icaridin: 260 (186-undefined) Figure 5.

CONCLUSIONS

Under laboratory conditions, the hydroxylated cyclic acetals mixture, as obtained from the condensation between glycerol and citronellal give a 99% protection efficacy against *A. albopictus* when applied on the skin at a dose of 16.67 $\mu g/cm^2$.

The citronellal derivatives maintained an efficacy against *A. albopictus* above 90% for 8 hours at 0.17 mg/cm^2, much longer than citronellal, reported to protect for less than 1 h from *A. aegypti*.[4] Under the same conditions, the effect of DEET dropped below 90% after 2 hours. When tested on the major malaria vector *A. gambiae* at a lower dosage (83 $\mu g/cm^2$), a 90% efficacy of citronellal derivatives lasted 7 h (PE=100% for 6 hours). The protection time of our products can be further increased using fixative components such as vanillin, a widely used additive known to potentiate the repellent effects of some essential oils.[8,37] In fact, an ethanolic solution containing 5% of citronellal derivatives with 1% of vanillin showed a protection efficacy higher than 98% for 8 hours against *A. albopictus*. An additional advantage of our citronellal acetals is their much weaker odour as compared to citronellal, and quite pleasant as judged by all volunteers involved in the study.

Because of their partial hydrosolubility, due to the presence of a hydroxyl group, the compounds could be easily formulated in hydroalcoholic solutions, which remain stable also when stored above room temperatures.

When tested in an area with high presence of *A. albopictus* using a latin square monitoring scheme, a 5% solution of citronellal derivatives remained active at 95% protection for 3 hours and at 85% protection for 6 hours. These values are lower than those measured with solutions of DEET and Icaridin, which however were tested at a fourfold higher dose.

Our results therefore indicate, as previously suggested[20], that the protection time of natural repellents can be improved by reducing their volatility and/or increasing their hydrophilicity, thus paving the way to new long-lasting insect repellents.

CONFLICT OF INTEREST DECLARATION

The authors declare that they have no commercial or financial relationships that could be construed as a potential conflict of interest.

ETHICAL STATEMENT

Volunteers agreed to take part in the experiments with an informed consent. This study has been approved by the Regional Ethics Committee for Clinical Trials of Tuscany Region with the registered number 20383_spe.

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AUTHOR CONTRIBUTIONS

II and FRD conceived the study. II, BC, PC and FRD participated in the design of the experiments and the interpretation of the results. II, BC, PC, AM and FRD performed the experiments. AM and MM made substantial contributions to acquisition, analysis and interpretation of data. FRD and II wrote the first draft of the manuscript. All authors read, corrected and approved the manuscript.

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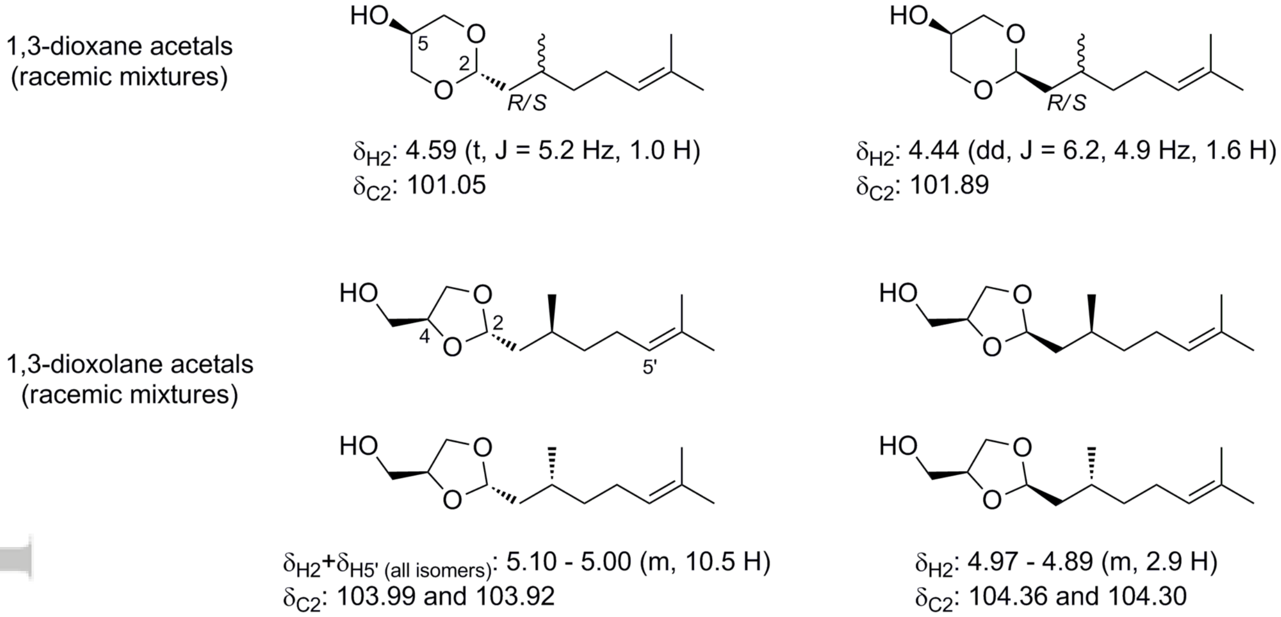

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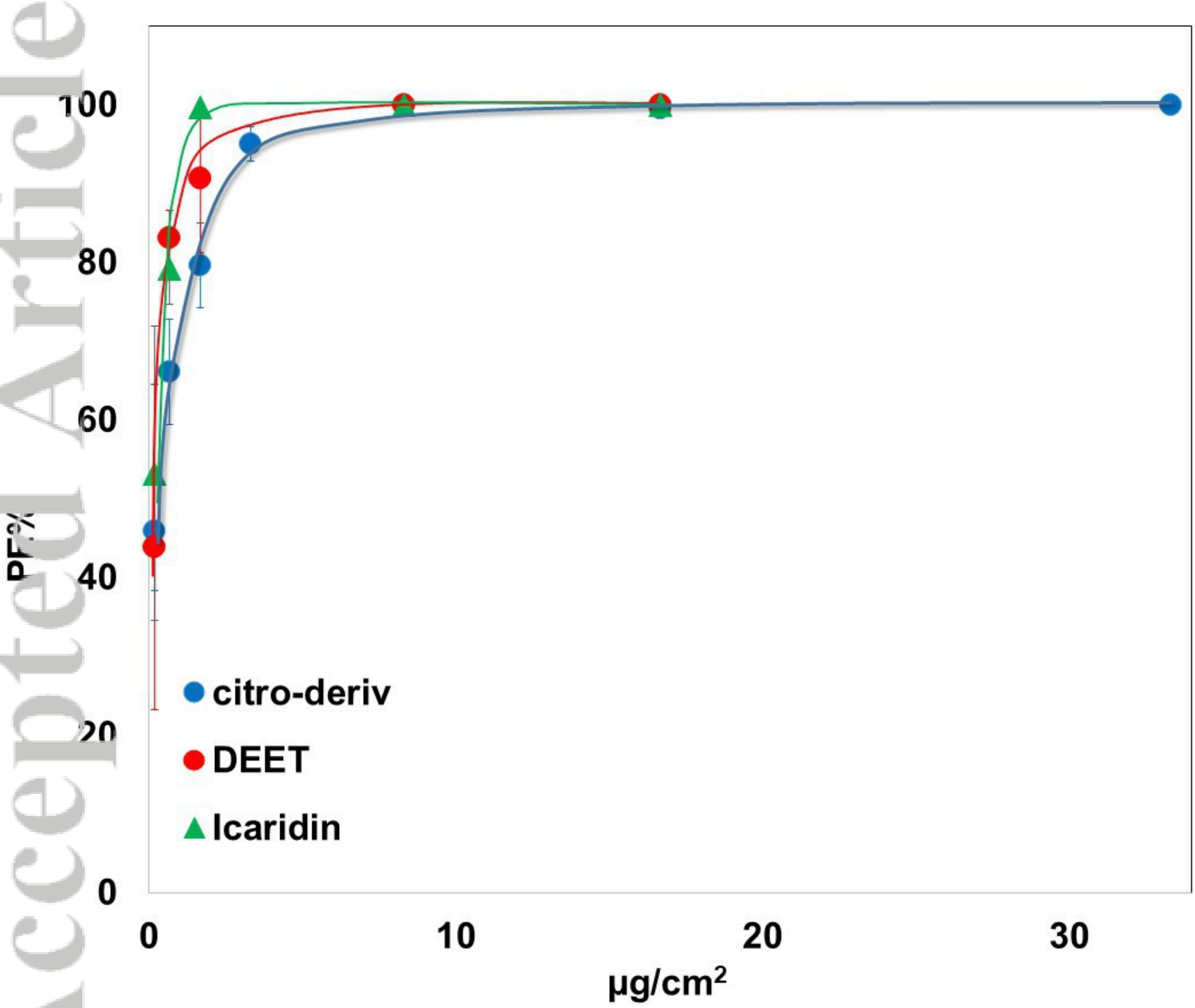

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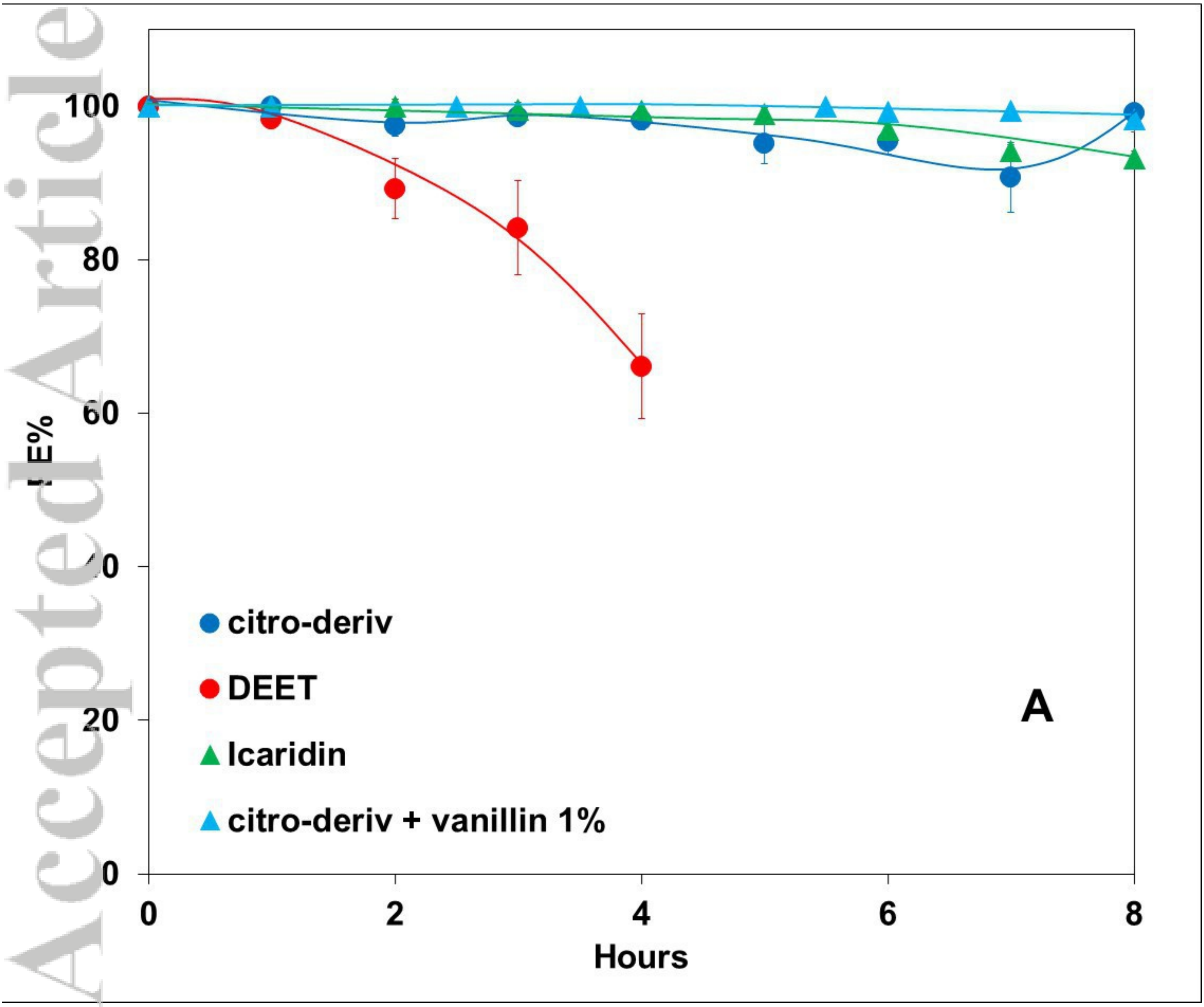

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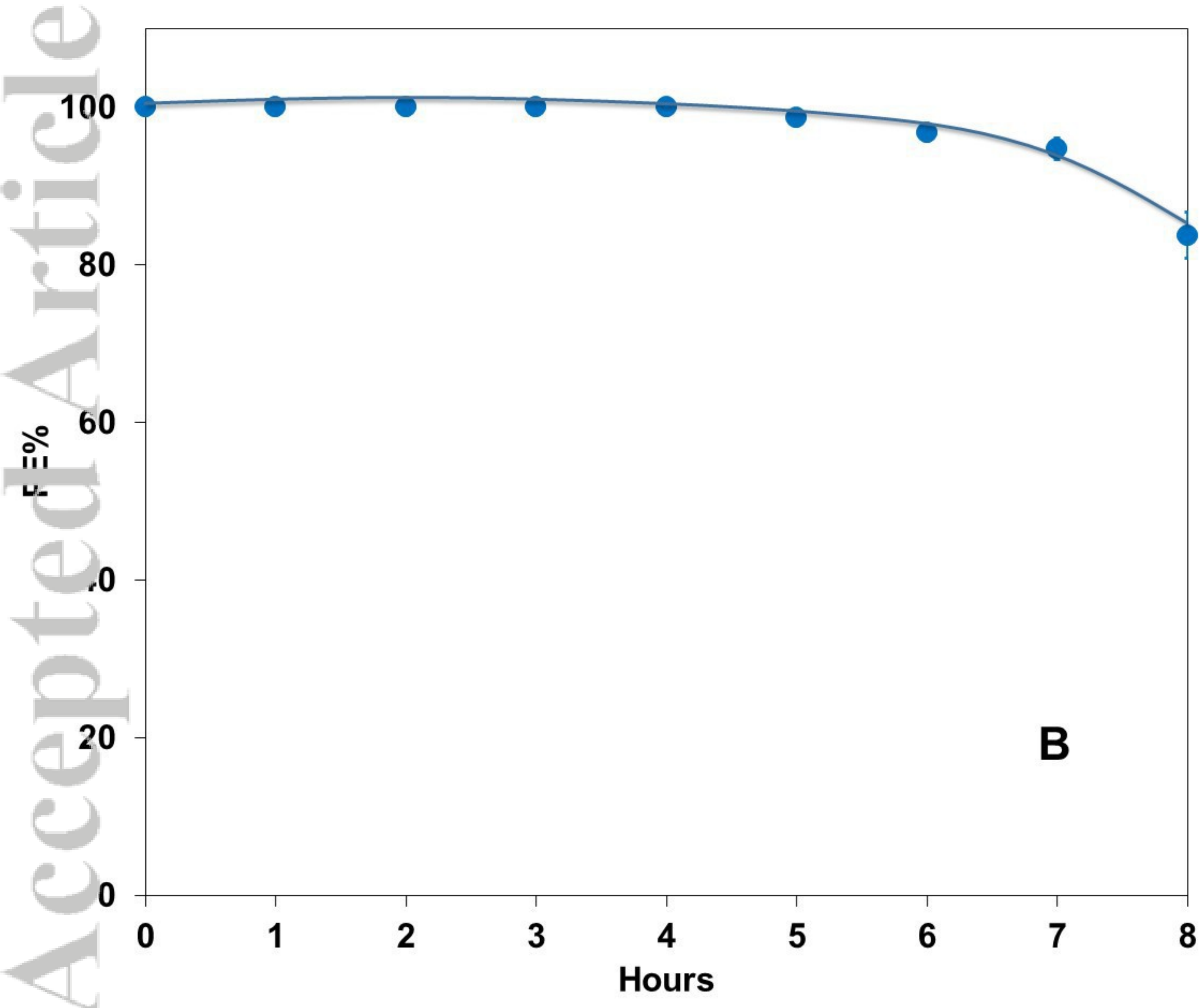

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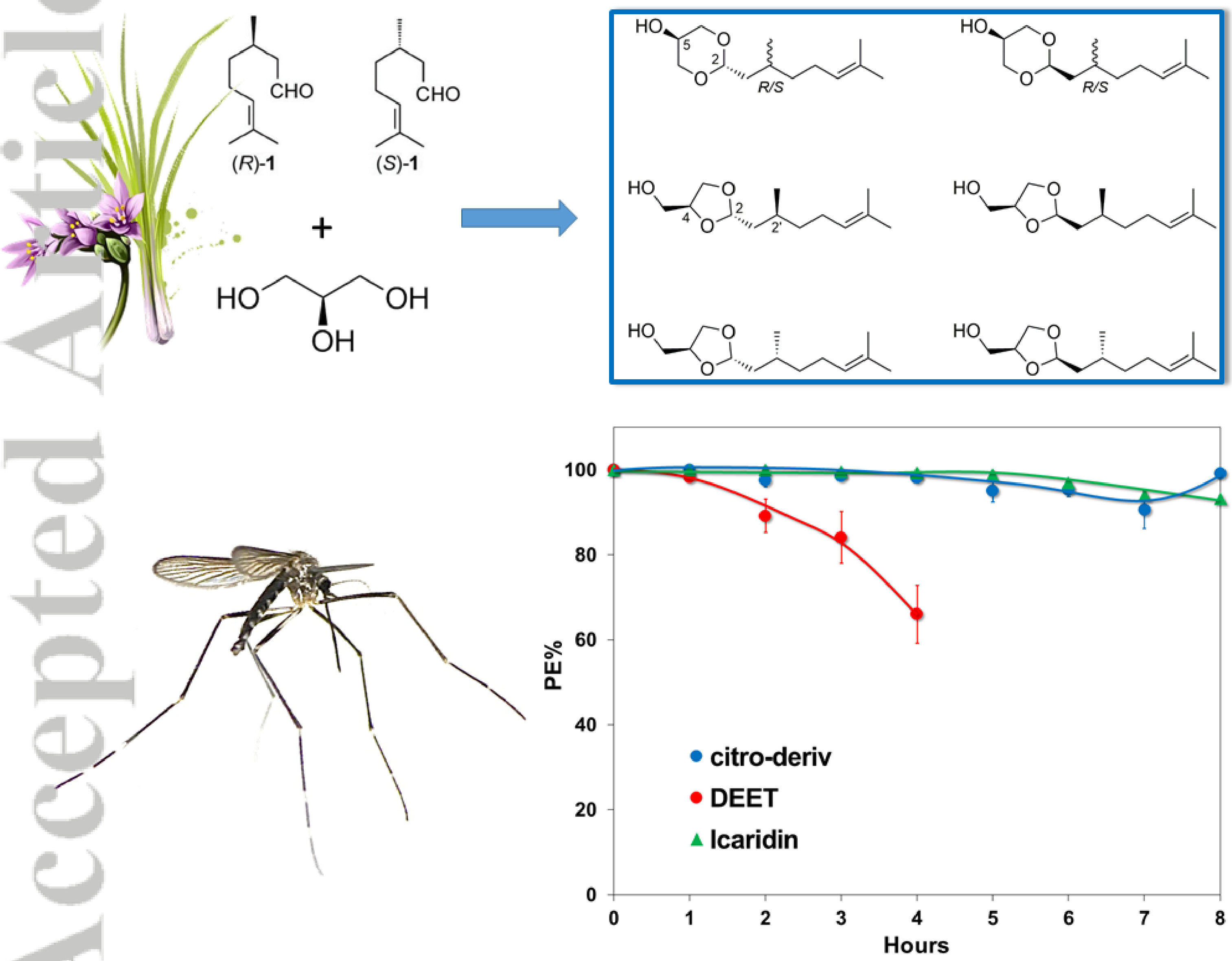
Graphical abstract

The mixture of hydroxylated cyclic acetals, obtained from condensation of citronellal with glycerol, was tested for its repellency against mosquitoes and showed a longer-lasting protection with respect to DEET.



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Time from start of trial	**Mean protection (95% Confidence Intervals)**		
	Citronella derivatives (5%)	*DEET (20%)*	*Icaridin (20%)*
Start	99% (98.2 – 99.5%)	100% (99.9 – 100%)	99.6 (98.9 – 99.9%)
Half-time (3.5 hours)	94.2 (92.9 – 95.3%)	99% (98.4 – 99.5%)	98.2 (97.4 – 98.8%)
End (7 hours)	79% (76.1 – 81.6%)	83.3 (80.5 – 85.8%)	93.8 (92 – 95.3%)

Table 1. Results of probit model assessing protection (defined as one minus the ration between the number of mosquitoes landing on treated skin and those landing on the controls) measured during the randomized design and blind field trial test with 4 volunteers in a temperate area with high presence of *Aedes albopictus*. Estimates reported are predicted probabilities at three time points: Start = at the beginning of the trial, Half-time = at 3.5 hours from the beginning of the trial, End = at 7 hours from the beginning of the trial.