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Cyclic voltammetry of immobilized particles as an alternative pesticides screening method for *aedes aegypti* mosquitoes

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Abstract: Today, pest control by chemical treatment is the main strategy against pest insects. This article proposes to show how cyclic voltammetry of immobilized particles could be applied in the context of pest research by screening several compounds known to have an activity against a pest model as *Aedes* species. *Aedes* mosquitoes are vectors of many diseases that represent a threat to humans such as yellow fever and dengue fever in tropical environments. In order to develop innovative screening approaches for new pesticides we search molecules that can interact potentially with a pest model (*Aedes* species). We characterized electrochemically the mosquitoes using voltammetry of immobilized particles (VIMP) method and analyze modifications on its characteristic signals in contact of insecticides.

Keywords: Pest, insecticides, *Aedes aegypti* mosquitoes, screening, voltammetry of immobilized particles (VIMP), cyclic voltammetry.

Introduction

The emerging resistance to insecticides is of primary concern. Pesticide discovery can follow two principal approaches. Assays performed on whole living insect and target-based approaches. The first ones have the advantage of allowing the discovery of drugs acting on unidentified targets but imply the use of insects alive. In another side, target-based approaches require much simpler equipment.

Between the different pest diseases, we choose the vector-borne ones as model for our study. Vector-

Technics	Benefits	Inconveniences
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borne diseases are responsible for over 17% of infectious diseases, and cause more than one million deaths each year [1]. In 2019, according to World Health Organization, malaria transmitted by *Anopheles* mosquitoes, affected more than two hundred millions of people causing more than four hundred thousand deaths worldwide [1, 2]. Yellow fever and dengue fever, transmitted both by *Aedes* mosquitoes, are responsible for thirty thousand and forty thousand deaths respectively, worldwide every year [1, 3]. Dengue fever is the most common viral infection transmitted by mosquitoes of the genus *Aedes*. More than 3.9 billion people in more than 129 countries are at risk of contracting dengue [1, 4]. Other diseases transmitted by mosquitoes include Chikungunya virus fever, Zika virus fever, West Nile virus fever, Lymphatic filariasis and Japanese encephalitis [1] as shown in table 1.

Table 1. Main mosquito vector-borne diseases

Mosquito genus vector	Transmitted diseases	pathogen agent
<i>Aedes</i>	Chikungunya	Virus
	Dengue	
	Lymphatic filariasis	Parasite
	Rift Valley fever	Virus
	Yellow fever	
	Zika	
<i>Anopheles</i>	Lymphatic filariasis	Parasite
	Malaria	
<i>Culex</i>	Lymphatic filariasis	Parasite
	Japanese encephalitis	Virus
	West Nile fever	

Vector-borne diseases are preventable through the application of protective measures and community mobilization. Chemical treatment is the main strategy against disease transmission. This strategy consists in finding new compounds having an activity on mosquitoes' behavior, using current techniques, then making products such as treated mosquito nets, insect repellents, coils and insecticide sprays [5] available to the population. Global reality shows that it is necessary to find new techniques and compounds to control these vectors. Targeting the olfactory apparatus may be a strategy to prevent from vector-borne diseases. In fact, several studies [6–8] have shown the importance of the role of olfaction in the orientation and behavior of insects for finding hosts and essential nutrients for survival, laying eggs, expressing social attributes and reproduction and avoiding enemies [7]. Many techniques have already been developed to study olfaction in insects [7]. Table 2 summaries their main advantages and inconveniences.

GC/GC-MS [7, 9]		Excellent ability to detect odors	Skilled operators needs
Electronic nose [7]		Small volume needs, High reliability, Quick response	Single use of gas phase samples
NIR spectrum technology [7]		High sensitivity Performing simultaneous analysis and measurement of a variety of gases	Impossibility of real-time detection
Biomimetic olfactory sensors	Microcantilever biosensor [7]	Small volume needs, Portability Easy assembly	Unstability in liquid medium
	Electroantennography (EAG) techniques for insects [7]	Providing guidance on attractants, lures and insect repellents	Undetermination of odorant molecules concentrations
	based on quartz crystal devices [10, 11]	High sensitivity Fast response speed Real time dynamic detection	
	based on field effect transistors (FETs) [7]	Quick response Very good selectivity	
	based on microelectrodes [7]	Allowing study of characteristics of the olfactory response of neurons	Inability to show actual action between cells and ligands
	based on light addressable potentiometric sensors (LAPSs) [7]	Easy flowing fluids measurements thanks to smooth surfaces Simple manufacturing Long service Addressable measurement at any location of the chip surface	
Electrochemical biosensor [7]		Simple instrumentation Easy to use Quick response	Lack of reproducibility
Olfactory receptor cell electrochemical biosensor [12–14]		Lifetime Stability Reproducibility Selectivity	Inability of the method to reflect the interaction between receptors

Table 2. Technics for studying olfaction in insects.

Moreover, many studies highlighted compounds that modify mosquito's behavior. Some of those compounds are repellents and others are used for deterrence [15]. N, N-diethyl-3-methylbenzamide (DEET) is one of the most used insect repellents having the ability to act as a repellent even in the presence of substances that attract mosquitoes [16]. This compound also deters laying for three different mosquito species *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus* [15]. Although its extremely effectiveness, as some mosquitoes become addicted to this substance [17], it is therefore necessary to find new compounds. Other market-shared products as Icaridin and IR3535, which present less toxicity in their composition, have been employed especially for children over 6

months old and pregnant women [18, 19] although DEET proved more effective [20]. Afify *et al.* have tested three non-toxic compounds, Methyl N,N-dimethyl anthranilate (MDA), ethyl anthranilate (EA) and butyl anthranilate (BA), as compared to DEET and showed the repellent behavior of MDA, EA and the deterrent behavior of EA, BA [15]. Some bioactive molecules such as isovaleric acid, myristoleic acid, myristic acid and oct-1-en-3-ol may have potential applications as they could be used as a mosquitoes' trap. In fact, among these compounds, the three acids constitute major compounds from larval extracts of *Aedes aegypti* species identified by Wang and colleagues [21] and oct-1-en-3-ol is known to be a positive control for *Aedes* mosquitoes attraction [5, 6, 16, 21–26].

For screening potential anti-mosquito's compounds, voltammetry of immobilized particles (VIMP) method can be a useful tool. This solid-state electrochemical technique introduced in the late 1980s by Scholz *et al.* [27–30], has become an increasingly growing research field because of its high sensitivity and the possibility of application to a variety of fields such as screening of pharmacological drugs [31], monitoring of plant defense against external stressors [32], identification and characterization of food dyes in food science [33] and other organic compounds in solid state [34–36]. This technique has been applied in the evaluation of solid-state electrochemical behavior of compounds, qualitative and semi-quantitative determinations, monitoring of products derived from specific reactions, authentication and discrimination of several samples, and as quality control analytical tool [34]. VIMP has the assets to have an easy-to-build analytical set-up and low costs, to perform quick analyses with small amount of samples. VIMP has also proved to be in supporting and integrating other analytical techniques such as chromatography, spectroscopy [31, 32] contributing to the identification of new compounds or in-depth study of reactions in various materials from organic applications.

In the present work, we used VIMP with *Aedes aegypti* mosquitoes as a screening method to determine if molecules among isovaleric acid, myristoleic acid, myristic acid, oct-1-en-3-ol and commercial repellent active molecules may have an interaction with mosquito's metabolites, and can be considered as a potential anti-mosquito (anti-pest) molecule. To best of our knowledge in the vector-borne disease field, cyclic voltammetry has been used for dengue virus protein analysis [37] but no electrochemical work using VIMP with mosquitoes for such screening have been found. For our experiments, we performed cyclic voltammetry (CV) in a mosquitoes methanolic extract solution and in solid deposition as electrochemical analysis coupled with classical gas chromatography coupled with mass spectroscopy (GC–MS) characterization to identify mosquitoes' metabolites. This study can be considered as a first step to the development of new target-based approaches for insecticidal activity studies.

Materials and methods

Chemicals

The chemicals used included: isovaleric acid and oct-1-en-3-ol (purity \geq 99%, Sigma Aldrich Inc., St-Louis, MO, USA), for positive controls, meaning compounds which generated an antennal response in the presence of mosquitoes during the electroantennographic analysis; myristoleic acid and myristic

acid (purity $\geq 99\%$, Sigma Aldrich Inc., St-Louis, MO, USA) for negative controls; tetrabutylammonium hydrogensulfate, NBu_4HSO_4 , (97%, Sigma Aldrich Inc., St-Louis, MO, USA) and methanol (purity $\geq 99\%$, Reag. Ph.Eur. ACS, VWR International, Fontenay-sous-Bois, France) for the electrolyte medium. Three commercial mosquito repellents were used containing 48.5% of DEET, 20% of Icaridin and 34.3% of IR3535 respectively.

Mosquitoes

A metapopulation of *Aedes aegypti* was established by sampling around private houses where the residents gave their permission for mosquito larvae collection. The sampling activities were conducted from July to August 2019 in the 5 following localities of Guadeloupe (French West Indies): Les Abymes, Pointe-à-Pitre, Deshaies, Saint-François, and Anse Bertrand. Experiments were performed on the 12th, 13th and 14th generation of this colony. Mosquitoes were maintained under laboratory conditions of $28 \pm 1^\circ\text{C}$ and a relative humidity of 40–60%. Larvae were reared at densities of 200–300 larvae/L in dechlorinated tap water and were fed rabbit pellets. Adults were given ad libitum access to a 10% sucrose solution. For electrochemical analysis, mosquitoes were frozen at -4°C until death. Cyclic voltammetry analysis was performed on male and female *Aedes aegypti* mosquitoes, on male mosquitoes' head, which was separated from the body using a microscope and a surgical blade, and on a mosquitoes extract solution.

Mosquitoes extract solution

The mosquitoes extract solution was prepared by placing 0.05g of *Aedes aegypti* male mosquitoes in 6mL of pure methanol. The mixture was stirred during 2h then filtered to remove mosquito's parts. Around 0.15 g of NBu_4HSO_4 were dissolved leading to a concentration of 0.07 mol.L^{-1} as supporting electrolyte, in the methanolic extract to obtain the final analytic extract solution.

Electrochemical methods

The parameters used for electrochemical analysis are similar to those chosen in the work of Domenech *et al.*[38]. Electrochemical experiments were performed at $298 \pm 1\text{K}$ in a thermostated cell with CH I660 equipment. A typical three-electrode arrangement was used: a BAS MF2012 glassy carbon working electrode (GCE) (geometrical area 0.071 cm^2), a platinum wire auxiliary electrode and an Ag/AgCl (3 M NaCl) reference electrode. The analytical medium is a 0.07 mol.L^{-1} NBu_4HSO_4 solution in methanol (MeOH). The electrochemical measurements on solid deposit were performed on mosquito's films by rubbing the lower end of the glassy carbon electrode over full-body or head mosquitoes sample and cleaned with a tissue paper in 6 mL of analytical medium. The classical electrochemical measurements was performed by immersing the three-electrode arrangement in the mosquitoes extract solution. Concentrations of isovaleric acid, oct-1-en-3-ol, myristoleic acid and myristic acid in the electrolyte solution were $10^{-1} \text{ mol.L}^{-1}$, $10^{-4} \text{ mol.L}^{-1}$, $10^{-1} \text{ mol.L}^{-1}$ and $10^{-3} \text{ mol.L}^{-1}$ respectively. Commercial pesticides were added to the electrolyte solution leading to a

pesticide concentration of 10^{-4} mol.L⁻¹ for the analysis. Cyclic voltammograms were recorded in the -1 to +1 V potential range initiating the potential scan at 0 V in the positive direction at a 20 mV.s⁻¹ scan rate.

GC-MS method

A Thermo Scientific GC-MS instrument (Trace 1310 GC and Exactive Orbitrap) operating in electronic ionization (EI) mode was used for the analysis. Helium 5.0 (Linde, France), He \geq 99.999% purity was used as carrier gas at constant flow of 1 mL.min⁻¹. A Thermo Scientific TG-5SILMS capillary column (silarylene) was used for separation. The GC as operated in splitless mode with surge and the injection volume was 1 μ L. Splitless time was set at 0.75 min, split flow at 30 mL.min⁻¹, septum purge was set at constant flow of 5.0 mL.min⁻¹. The injector temperature was 280°C, the transfer line was 320°C, then 300 °C (GC) and finally 280 °C (MS). The initial GC oven temperature of 40°C was held for 1 min, and then increased at a rate of 20°C.min⁻¹ until the final temperature of 120°C was attained. Then increased at a rate of 5°C.min⁻¹ until the final temperature of 310°C was attained. Then increased at a rate of 10°C.min⁻¹ until the final temperature of 325°C was attained. Finally increased at a rate of 20°C.min⁻¹ until the final temperature of 345°C was attained. The time of the run was 46.5 min. The emission current was 50 μ A and the electron energy was 70.0 eV. Tune and calibration was performed with perfluorotributylamine (FC 43), before each sequence. For identification, the following ions were monitored from full-scan mass spectrum. Xcalibur software was used for data acquisition and data were processed using Thermo Scientific™ TraceFinder™ 5.0 software (deconvolution and identification of unknown substances). First, the peaks were deconvoluted to obtain pure mass spectra using the Deconvolution Plugin of the software. The spectra of the unique peaks in the sample were then compared with standard spectrum library NIST 2017 (2.3 version) and in-house spectral libraries. After the deconvolution process, the results were filtered using the accurate mass information and by comparison of spectra from the libraries. So, a list of substances was established according to the comprehensive scores by using RHRF and RSI scores and Δ RI. RHRF score is the percentage of the number of ions in the spectrum libraries that can be explained by mass peaks in the total ion chromatogram of the sample spectrum. RSI is the reverse search index score for each compound detected returned by the libraries search. Δ RI is the deviation of retention index. Therefore, results are reliable when HRF and SI values are high and Δ RI is low.

Before sample analysis, the mixed solution of C8-C40 alkanes calibration standard was analyzed using the same separation method to determine the retention time of each n-alkane, which was used to calculate the retention index (Kovats Index, RI) of unknown substances. The deviation of retention index, Δ RI, was finally calculated by comparing it with the retention index of compounds included in the libraries.

Results and discussion

Mosquito's samples without pesticides: electrochemical analysis

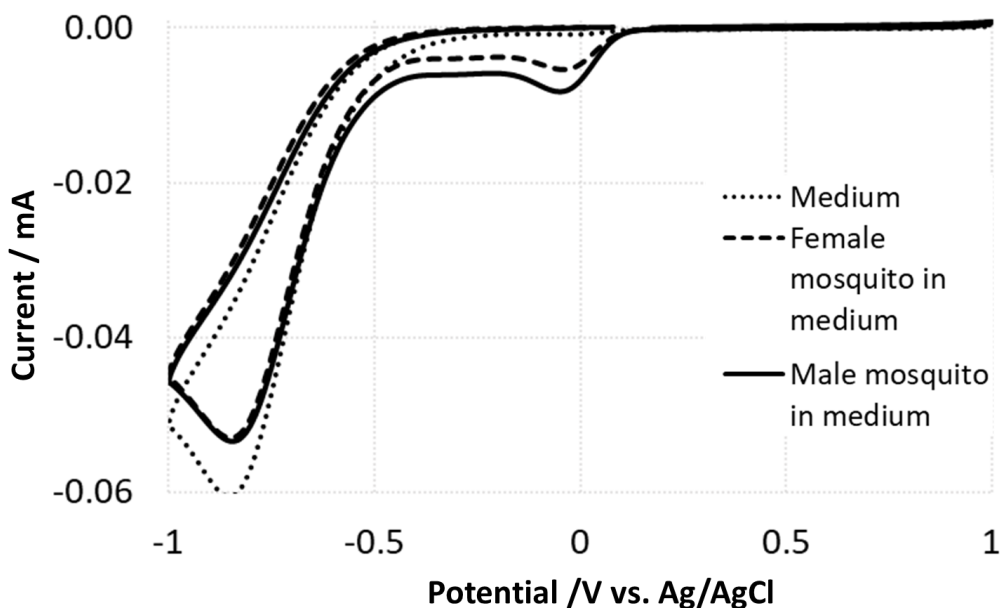


Figure 1. Cyclic voltammograms of female mosquito's full body deposit (dash line) and male mosquito's full body deposit (solid line) onto glassy carbon electrode, in contact with air-saturated $0.07 \text{ mol.L}^{-1} \text{ NBu}_4\text{HSO}_4$ solution in MeOH. Potential scan rate is 20 mV.s^{-1} .

Figure 1 represents cyclic voltammetry measurements obtained in the electrolytic medium without and with female and male mosquitoes. All curves present a huge peak at around -0.8 V which characterize the oxygen reduction reaction forming a superoxide ion in aprotic organic solvents [39] as shown in equation 1.



In the presence of weak Brönsted acids such as H_2O , the radical hydroperoxide is formed [40] according to equation (2). This species rapidly disproportionate in the presence of protic substrates into O_2 and H_2O_2 (equation 3):



In fact, experiments were performed in natural conditions with a certain amount of dissolved oxygen in the methanolic medium. This specific peak was also observed in the work of Jiang and Li [41] and elsewhere [39, 42, 43]. In both mosquitoes' curves, a broad reduction peak around -0.1 V is observed followed by a plateau spreading out to -0.5 V . Both genders seem to present the same electroactive molecules. The difference in intensity in the cathodic peaks can be explained by different amount of the sample mosquito transferred onto the electrode. In fact, indeed, repeatability/reproducibility measurements show that the peaks are located at the same potential but have variable absolute intensities (Figure S1).

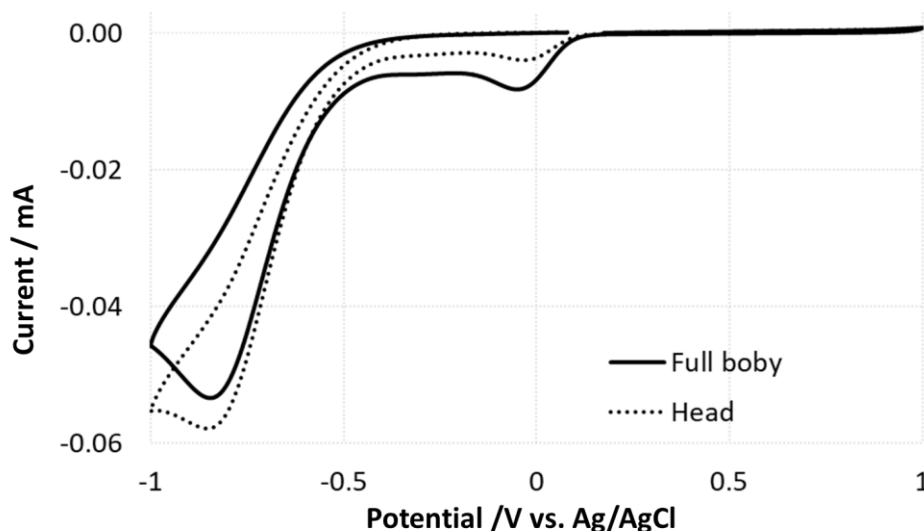


Figure 2. Cyclic voltammograms of mosquito's head deposit (dot line) and mosquito's full body deposit (solid line) onto glassy carbon electrode, in contact with air-saturated 0.07 mol.L^{-1} NBu_4HSO_4 solution in MeOH. Potential scan rate is 20 mV.s^{-1} .

Figure 2 shows cyclic voltammetry measurements obtained with mosquitos' full body and head. The two curves present the same shape with the signal at -0.1 V followed by the plateau. We may say that the electro-active molecules of the mosquito's head are therefore identical to those of the rest of the body. The difference in signal intensity may be due to the small amount of material that a mosquito head represents.

To identify the electroactive molecule of the mosquito, we compared the CVs of the solid deposition and the methanolic extract as shown on Figure 3.

A difference of 70 mV can be observed between the signal of the mosquito molecule in the liquid phase, at -0.12 V , and in the solid phase, at -0.05 V . This shift is not surprising in cyclic voltammetry when comparing the signals of organic molecules in solid deposit and in solution, the shape of the curves being sometimes very different [44] considering the film/electrolyte/base electrode three phase boundary [45, 46]. Moreover, the intensity ratio between the peak at -0.1 V and -0.8 V is higher in the solid deposit than in methanolic solution. This indicates that the catalytic efficiency of pyrazine (and other possible compounds with electrocatalytic activity in O_2 reduction) is higher in the solid phase than in solution.

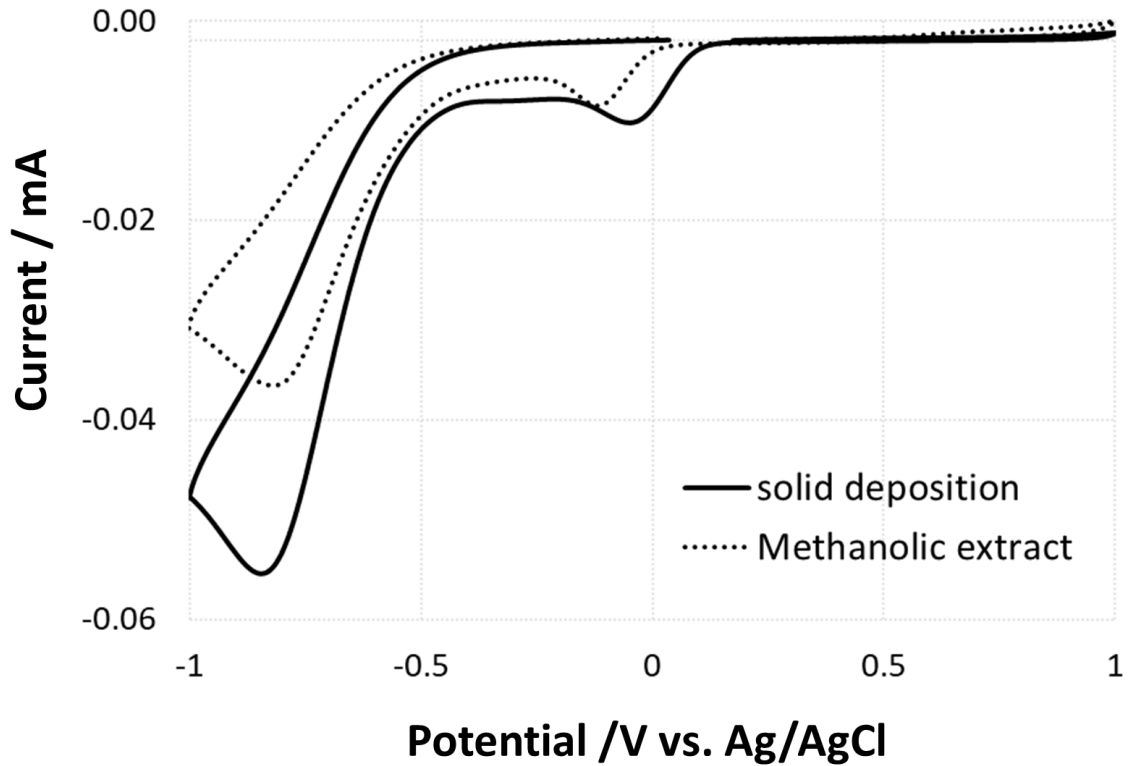


Figure 3. Cyclic voltammograms of mosquito's full body deposit (solid line) onto glassy carbon electrode in contact with air-saturated 0.07 mol.L^{-1} NBu_4HSO_4 solution in MeOH and mosquitoes air-saturated methanolic extract (dot line). Potential scan rate is 20 mV.s^{-1} .

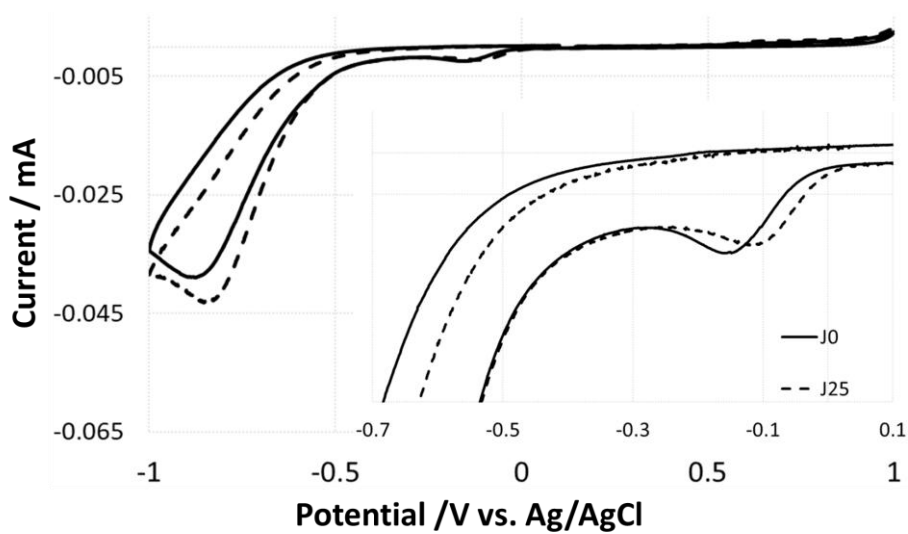


Figure 4. Cyclic voltammograms of mosquito's air-saturated methanolic extract obtained the death day (solid line), and twenty five days after death (dash line). Potential scan rate is 20 mV.s^{-1} .

Figure 4 presents cyclic voltammetry measurements obtained at zero and twenty five days after mosquitoes' death with, in insert, a zoom in electroactive species of mosquitoes. The intensity of the peak around -0.1V and the intensity ratio between the pyrazine and oxygen reduction signals remains similar any days after death. It seems that this electroactive molecule is stable after the death of the mosquito in the case that mosquitoes are kept cold between each analysis in order to study compounds that can interact with these electroactive molecules.

Mosquitos' samples without pesticides: GC-MS analysis

Table 3 presents a selection of metabolites identified results of the preliminary analysis of the chemical composition of mosquito's extract. The identification was done by comparison with GC-MS library in the mosquito's methanol extract. Between the identified compounds, several natural pyrazines, fatty acids and γ -sitosterol were found. Only compounds showing total scores above 85 % and retention index (RI) values close to that reported in databases according to a semi-standard non-polar chromatographic column were considered. Following this identification, the mosquito's characteristic peak may be attributed to pyrazine derivatives reduction reaction in non-aqueous solvents as shown in equation 4.

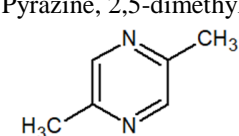
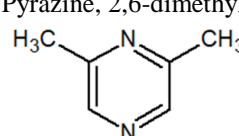


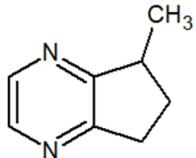
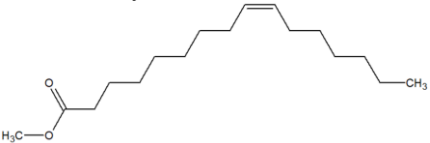
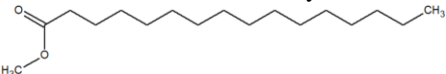
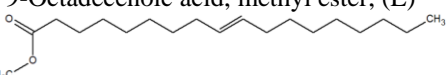
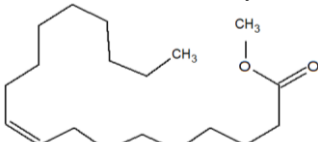
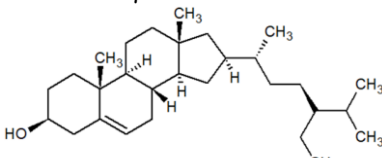
H^+ protons may be provided by fatty acids presents in mosquitoes' metabolites.

This type of molecules have been studied electrochemically in aqueous media [47–49] and, in general, the redox process in organic compounds involves protonation/deprotonation and it can produce intra- and intermolecular restructure by hydrogen bonding [50].

Focusing on natural pyrazines, these compounds are described in insects and their activities are related with alerting signals and insects' behaviour [51].

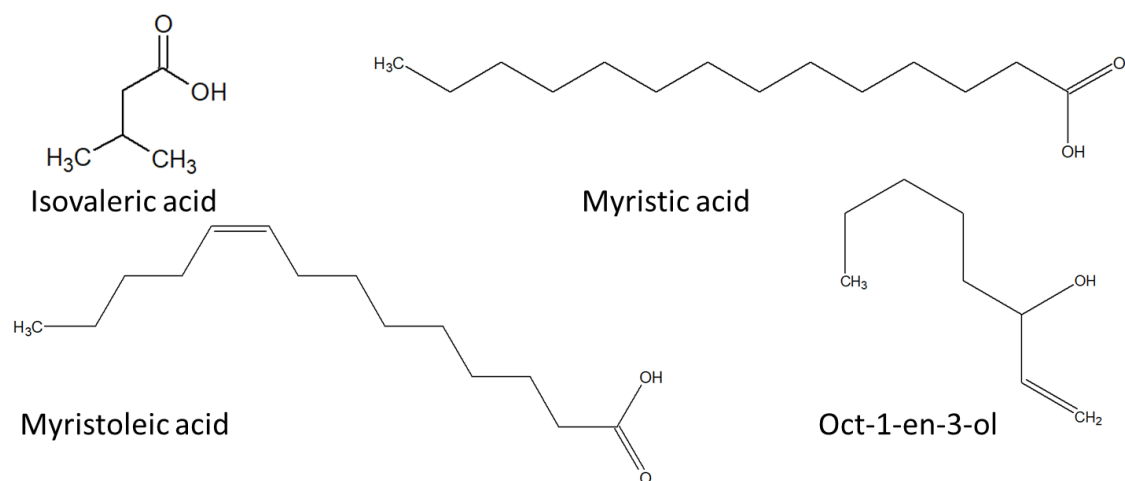
Table 3. Metabolites identified by GC/MS analysis

Component Name	Calculated Retention Index	Library Retention Index	Retention Time (min)	Reference m/z	Area (a.u.)	Total score
Pyrazine, 2,5-dimethyl- 	903	917	4,5	108,068	8003724	96,9
Pyrazine, 2,6-dimethyl- 	929	917	4,8	108,068	34798029	96,6

5H-5-Methyl-6,7-dihydrocyclopentapyrazine 	1158	1149	7,0	119,060	2377813	98
Methyl hexadec-9-enoate 	1911	1904	20,9	81,069	72299026	96,5
Hexadecanoic acid, methyl ester 	1933	1926	21,3	87,044	211079484	95,6
9-Octadecenoic acid, methyl ester, (E) 	2107	2110	24,6	81,069	96151768	96,7
13-Octadecenoic acid, methyl ester, (Z) 	2113	2126	24,7	81,070	4486443	95,9
γ -sitosterol 	3336	3321	42,2	145,101	23363032	96,9

Mosquito's samples with pesticides: electrochemical analysis

a)



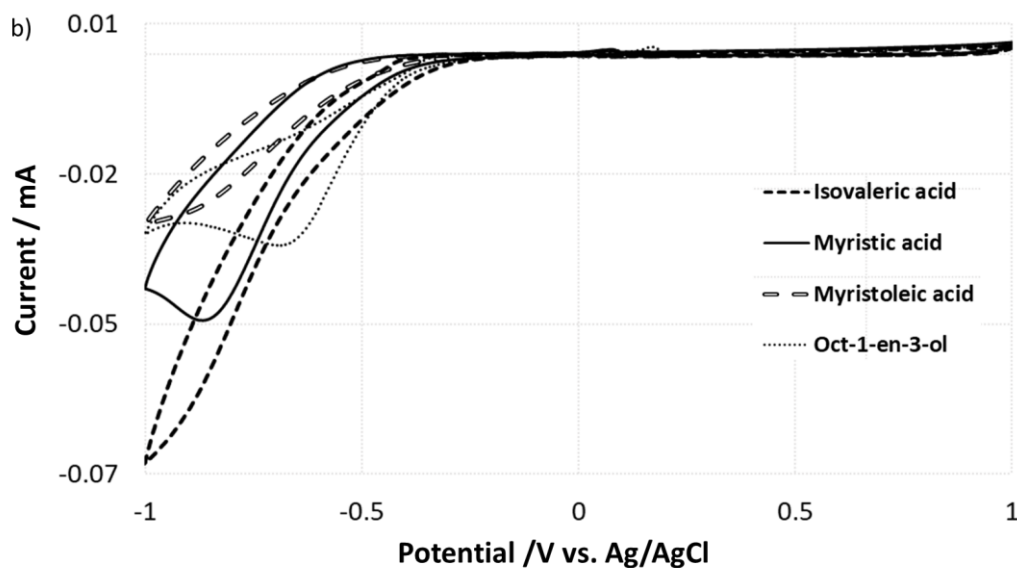


Figure 5. a) The compounds topologic formula b) Cyclic voltammograms of different compounds: isovaleric acid (dash line), myristic acid (solid line), myristoleic acid (empty dash line) and oct-1-en-3-ol (dot line) in air-saturated 0.07 mol.L^{-1} NBU_4HSO_4 solution in MeOH. Potential scan rate is 20 mV.s^{-1} .

Figure 5 shows cyclic voltammograms of positive controls, isovaleric acid (dash line) and oct-1-en-3-ol (dot line), and negative controls, myristic acid (solid line) and myristoleic acid (empty dash line). The oxygen peak shape at -0.8 V is modified in presence of these compounds. Isovaleric acid and myristoleic acid (highest concentrations) induce a strong changes in the oxygen reduction peak compared with oct-1-en-3-ol and myristic acid, which are presents at a lower concentration. This effect may be explained by oxygen reduction reaction involving proton-donor species in non-aqueous solvents as shown in equations 5 and 3 [43, 52] as explained before in the case of protic solvents.



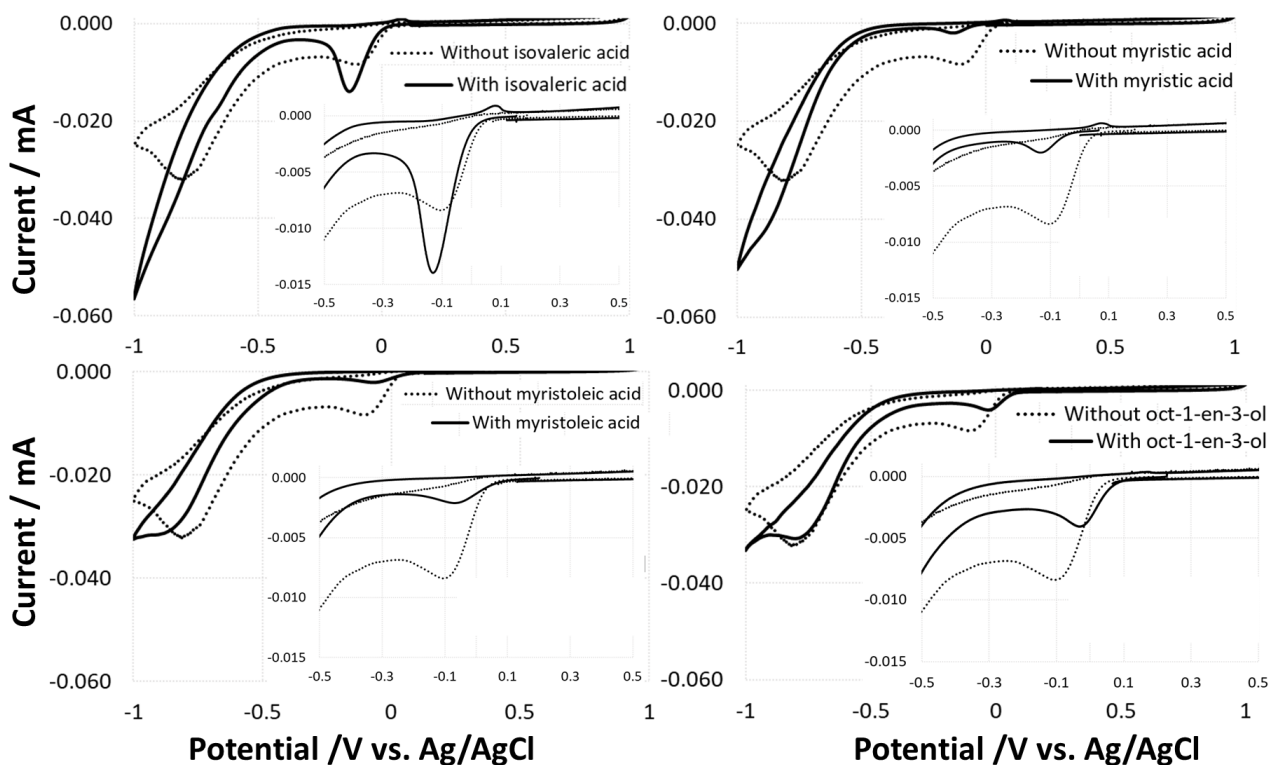


Figure 6. Cyclic voltammograms of mosquito's full body deposit onto glassy carbon electrode, in contact with air-saturated $0.07 \text{ mol.L}^{-1} \text{ NBU}_4\text{HSO}_4$ solution in MeOH without (dot line) and with (solid line) the different compounds. Potential scan rate is 20 mV.s^{-1} . In insert, a zoom on the region from -0.3 V to 0.3 V .

Figure 6 presents cyclic voltammograms of mosquito's full body deposit onto glassy carbon electrode obtained without and with positive controls, isovaleric acid and oct-1-en-3-ol, and negative controls, myristic acid and myristoleic acid. The mosquito's characteristic signals located at -0.1 V is identified in all cyclic voltammograms. The ratio between the intensity of the peak at -0.1 V and the plateau intensity is higher in presence of isovaleric acid. In the presence of myristic acid, the mosquito specific signal at -0.1 V significantly decreases. For both molecules, this mosquito cathodic peak is accompanied, in the reverse scan, by a weak anodic peak at ca. $+0.05 \text{ V}$, as seen in Figure 5. Cyclic voltammograms obtained with myristoleic acid and 1-octen-3-ol show also a significant decreasing of the mosquito cathodic peak intensity at -0.1 V without an associated anodic peak in the reverse scan. The presence of the weak anodic signal may be assigned either to the oxidation of isovaleric acid and myristic acid in excess or to a new oxidizable compound resulting from a reaction between the mosquitoes molecules and the acids (for example, a chemical reaction with pyrazine).

Isovaleric acid is a positive control because of its antennal response during electrophysiological tests [23]. Behavior studies have shown that this compound, in high doses, informs females that a site is unsuitable for breeding. The presence of isovaleric acid indicates high bacterial activity in the breeding site [23]. Those results may indicate that the antennal response is described by a modification of relative intensity of the mosquito's electrochemical signals. Previous research has shown that myristic acid does not influence mosquito oviposition and does not elicit an antennal response [23]. The decreasing in the electrochemical signal of mosquitoes in the presence of myristic acid could indicate

that this compound which interact with the mosquito's electro-active molecules does not necessarily have a specific activity against the mosquito. Myristoleic acid is considered as a negative control. It is a cytotoxic compound that acts as a plant metabolite, apoptosis inducer [53] and carboxylesterase inhibitor [54]. In the literature, myristoleic acid deters mosquito laying. However, during electroantennographic experiments, this compound does not elicit an antennal response [23]. Myristoleic acid may have an interaction with the mosquito, and this interaction could be a cause of the deterrent behavior. Electrophysiological tests and behavior studies have shown that 1-octen-3-ol is a positive control [16, 17]. This molecule is used in several studies on mosquito olfaction. It acts as an attractant for insects [22] and is used for their capture. Results presented on these CVs curves may be an indication of a modification of the mosquito's electrochemical signals.

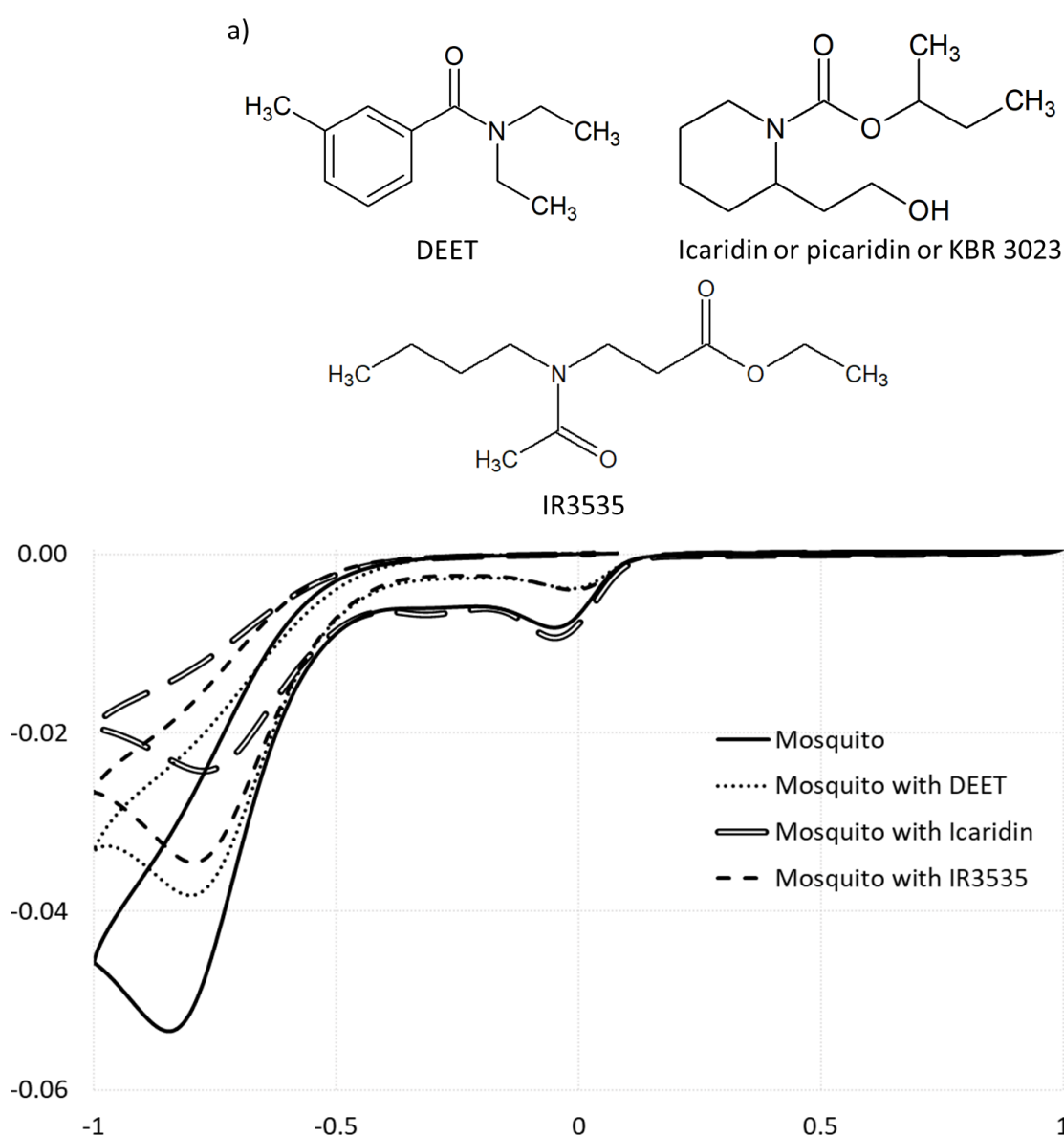


Figure 7. a) The commercial compounds topologic formula b) Cyclic voltammograms of mosquito's full body deposit onto glassy carbon electrode, in contact with air-saturated 0.07 mol.L⁻¹ NBu₄HSO₄ solution in MeOH without (solid line) and with the different repellent

compounds: DEET (dot line), Icaridin (dash line) and IR3535 (empty dash line). Potential scan rate is 20 mV.s⁻¹.

Figure 7 represents the topological formulas and the cyclic voltammetry curves obtained from mosquitoes in the presence of marketed molecules in mosquito repellents. It can be seen that, as myristic acid, myristoleic acid and oct-1-en-3-ol, the intensity of mosquito's characteristic peak decreases with DEET and IR3535 which may explain their repellent character. Unlike the other two commercial molecules, Icaridin seems to have no effect on the mosquito's electroactive molecules.

Conclusion

We worked on the development of a CV-based screening method for the detection of compounds active against the *Aedes aegypti* mosquitoes. An unidentified mosquito's electroactive molecule (or pool of molecules) displays a significant signal at around -0.1 V, in solid and liquid phase.

A preliminary analysis of the chemical composition of the mosquito was done. Several natural pyrazines, fatty acids and steroids were identified by GC/MS. The confirmation of the identification is currently in progress.

Compounds such as 1-octen-3-ol and isovaleric acid, considered positive in electrophysiological tests, seem to show a modification in the CV signals characteristic of the mosquito. Myristoleic acid is a special case because it is inactive in electroantennography and positive in mosquito behavioral tests. This compound modifies the characteristic CV signals of the mosquito and according to our criteria is positive for our screening. Analysis with commercial repellents showed the effect of DEET and IR3535 on mosquito's signal compared with Icaridin. The results obtained during this preliminary study show the potential of CV for the development of alternative screening methods for the research of new pesticidal molecules.

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Supporting information

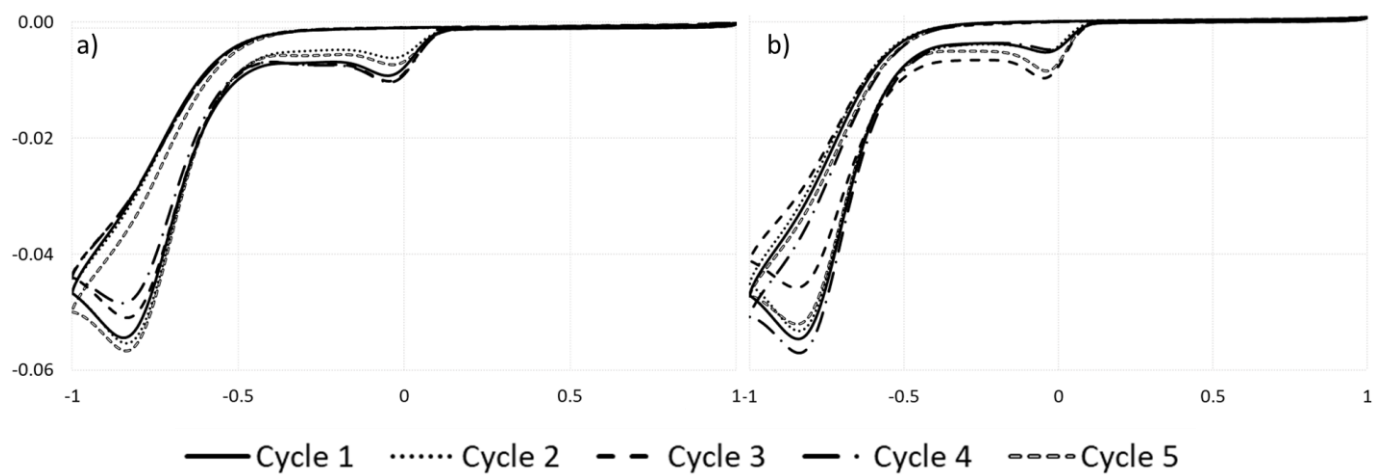


Figure S1. CV with a potential sweep from a) 1 V to -1 V and b) -1 V to 1 V.