The excito-repellent activity of five essential oils extracted from local plants against dengue and malaria vectors in Burkina Faso

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Abstract

Using chemical insecticides is the main way to prevent and manage dengue and malaria, two main mosquito-borne diseases. However, the vectors of these diseases have developed resistance to chemical insecticides. Essential oils (EOs) could be used as alternatives because besides their larvicidal and adulticidal properties, they have repellent properties. In Burkina Faso, studies on the repellent properties of essential oils on mosquitoes remained limited.

The EOs extracted from five plants (*Cymbopogon citratus*, *Cymbopogon nardus*, *Eucalyptus camaldulensis, Lippia multiflora*, and *Ocimum americanum*) collected in Ouagadougou were tested on populations of *Aedes aegypti* and *Anopheles gambiae* s.l collected in Bobo-Dioulasso and the Kou of Valley, respectively. Susceptible strains of the two species were also tested. DEET and permethrin were used as positive controls. Most of the 5 EOs tested produced irritant effects on adults of *Ae. aegypti* and *An. gambiae*. The irritant effects on mosquito adults were significantly influenced by the concentration of the EOs and species of mosquitoes. On *An. gambiae* s.l, the repellent-irritant effects of *C. citratus*, *C. nardus*, and *E. camaldulensis* EOs were very close to that of DEET. On *Ae. aegypti*, all our EOs had higher repellent-irritant effects and some of them were very close to DEET. EOs could be used as alternative repellents to pyrethroids in vector control.

Introduction

Global economic development increased urbanization, and climate change have significantly increased the mosquito-borne disease transmission pattern and dynamics. (Anoopkumar & Aneesh 2022). Mosquito-borne diseases are those spread by infected mosquito bites. Among the major vector-borne diseases, there are Zika, West Nile fever, Chikungunya, dengue, and malaria (WHO 2020).

The use of chemical insecticides is one of the main components in preventing and managing these mosquito-borne diseases (Van den Berg et al. 2021). Repellent sprays, indoor use of insecticides, or the wide distribution of impregnated mosquito nets are always limiting their proliferation (Bhatt et al. 2015; Rubert et al. 2016; Darriet 2007). Unfortunately, mosquitoes, particularly dengue and malaria vectors have developed resistance strategies to chemical insecticides (Diabaté et al. 2002; Namountougou et al. 2012, 2019, 2020; Ouattara et al. 2019). Regarding several environmental and health risks associated with chemical insecticides (Isman 2006) and the threat of mosquito resistance to these insecticides, essential oils (EOs) are being used as alternative control methods (Bossou et al. 2013; Gnankiné and Bassolé 2017; Deletré et al. 2013). Most of the studies have shown different properties of EOs: larvicidal properties (Bassolé et al. 2003; Sarma et al. 2019; Balboné et al. 2022a), adulticidal properties (Wangrawa et al. 2019; Yameogo et al. 2021, Balboné et al. 2022b,c), feeding deterrence (Hummelbrunner and Isman 2001; Blackwell et al. 2003) and repellent properties (Deletre et al. 2016). Although many studies have been carried out on the properties of essential oils in Burkina Faso, little is known about the repellent or irritant properties of essential oils on mosquitoes in general and particularly in western Burkina Faso.

EOs are odorous products obtained by steam distillation of specified botanical materials and separated from the aqueous phase by physical processes (Baptista da Silva et al. 2020). Their composition can vary considerably among species and varieties of herbs and within the same variety from different geographical areas (Zygadlo et al. 1993; Abena et al. 2007).

Repellents are products that work locally or remotely to dissuade an arthropod from landing on, flying towards, or biting human or animal skin (or any surfaces in general) (Blackwell et al. 2003; Choochote et al. 2007; Duvallet et al. 2012)

A large number of EOs extracted from different plant families have been found to have a high repellency against arthropod pest species. Among these EOs, *Cymbopogon* spp, *Eucalyptus* spp, *Lippia* spp, and *Ocimum* spp have been tested on mosquito species to assess their repellent properties (Tawatsin et al. 2001; Oyedele et al. 2002; Omolo et al. 2004; Dekker et al. 2011; Deletré et al. 2013). Studies have shown that permethrin and DEET, two chemicals, are repellents (Deletre et al. 2016; Badolo et al. 2004). Five repellent phenomena have been identified (Deletre et al. 2016): repellent-expulsion, repellent-masking, repellent-irritation, repellent-anti-appetizing, and visual repulsion. A repellent-expulsion is a substance that acts at a distance, causing the insect to flee from the stimulus (WHO 2013; Bernier et al. 2006). A masking repellent is defined as a volatile inhibitor that inhibits host search and location (Nolen et al. 2002). An excito-repellent called "landing inhibitor" according to WHO (2013), causes a flight movement towards the stimulus after physical contact with it (Deletre et al. 2016). Anti-appetant repellents corresponding to a disruption of feeding activity by contact with or ingestion of a chemical (Koul 2008).

The main objective of our study was to perform bioassays on *Ae. aegypti* from Bobo-Dioulasso and *An. gambiae* from “Vallée du Kou”, to evaluate the potential contact irritant effects of EOs of five plants (*Cymbopogon citratus*, *Cymbopogon nardus*, *Eucalyptus camaldulensis, Lippia multiflora*, and *Ocimum americanum*) and to compare these contact irritancy effects to those of DEET (N, N-diethyl-m-methyl benzamide) and permethrin’s positive controls.

Methods

Acquisition of essential oils

The EOs obtained from *O. americanum*, *C. nardus*, *L. multiflora*, *E. camaldulensis*, and *C. citratus* were obtained from the “Institut de Recherche en Sciences Appliquées et Technologies” (IRSAT) by hydrodistillation (HD) using a *Clevenger*-type apparatus and stored in a dark glass bottle at 4°C prior to use.

The procedures for gas chromatography/flame ionization detector (GC/FID) and gas chromatography/mass spectrometry (GC/MS) analyses have been described in our recent studies (Balboné et al. 2022a).

Mosquito Collecting And Rearing

Irritant repellency tests were performed on *An. gambiae* females from the Vallée du Kou (VK) strain (11º 23' 59" N, 4º 25' 46" W) and *Aedes aegypti* from the Bobo-Dioulasso strain (11° 10' 37.7" N, 4° 17' 52.4" W). Mosquito larvae of *An. gambiae* and *Ae. aegypti* collected in VK and Bobo-Dioulasso, respectively, were reared in the insectarium of the "Institut de Recherches en Sciences de la Santé/Direction Régionale de l'Ouest" (IRSS/DRO). They were fed with fish food. Emerging adults were placed in cages and fed with a 10% glucose solution. The females used for the bioassays were from batches of mosquitoes 3 to 7 days old and not fed blood.

Contact irritancy assays with WHO tubes test kit

The technique of impregnating Whatman papers with HE has been described by Balboné et al (2022b). The irritant-repellent test procedure of Grieco et al. (2005) set up for *Ae. aegypti* was adapted for *An. gambiae*. Batches of 20 non-blood-fed female mosquitoes aged 3 to 7 days were introduced into the tube containing the impregnated paper. After 30 seconds of exposure, the drawer separating the treated tube from the tube containing the neutral paper was opened. The mosquitoes were then left free for 10 minutes. Mosquitoes moving from the treated to the untreated tube were considered "escaped". Conversely, mosquitoes that remained in the treated tube were considered to be "left behind" mosquitoes. After the drawer was closed, the number of mosquitoes "escaped" and "remained" in each tube was counted.

Among the remained mosquitoes some individuals were knocked-down. The test was validated when the proportion of "escaped" mosquitoes in the control was less than 50%. The contact irritancy effect of a product is assessed based on the proportion of mosquitoes that escape. Knocked-down mosquitoes have been also recorded.

Data analysis

The proportions of escaped or dead mosquitoes were corrected by the Achee et al. (2009) control test values using the Abbot (1925) formula. The data were entered in Excel 2010. Data analysis was performed using XLSTAT software. The proportions of escaped mosquitoes were compared using Fisher's exact test. Essential oils and the two positive controls were then grouped according to the similarity of their effects using a hierarchical ascending classification (HAC) based on Ward's algorithm. This resulted in a binary segmentation tree, reflecting the hierarchy of similarities between the responses to the different products tested. The optimal number of classes in the tree was determined by decreasing the inter-class variance (branch height).

Results

Chemical composition of essential oils

For the five EOs, the chemical constituents and their relative percentage amounts determined by GC-MS analysis had been reported previously by Balboné et al. (2022a).

Contact Irritancy Assays

The contact irritancy effect was significantly different according to the strain of the mosquito and the product tested. Also, it was dose-dependent. Overall, in *An. gambiae* populations, the EOs tested showed a contact irritant effect (escape rate above 30%). It was not the case for *Ae. aegypti* populations.

The EOs of the five plant extracts as well as the two synthetic chemicals, DEET and permethrin, showed significant contact irritancy effects but at low concentrations for the EOs and high concentrations for chemicals (Figs. 1–4). In all mosquito populations tested, the HAC was summarized in 3 classes of response according to the similarity of the mosquito behavioral response (Figs. 1–4).

In An. gambiae populations

On the susceptible strain of *An. gambiae* "Kisumu", class 1 included *C. citratus* EO and DEET exhibiting escape rates of 50% at the lowest concentration (0.1%) and escape rates of 84.63 and 85% at the 0.5% concentration, respectively. At the highest concentration, *C. citratus* EO was less irritant (31.16% escaped) in comparison with DEET (56% escaped) while knock-down (KD) effects were high in the remaining mosquitoes. (Table 1). Class 2 includes *C. nardus* and *O. americanum* EO which were significantly more irritant at low concentrations and their activities decreased as concentration increased (60.05 and 51.88% at 0.1% concentration, 32.27 and 40.53% at 0.5%, 19.76 and 36.82% at 1%, respectively).

At the same time, these two EOs produced increasing KD effects with respect to the concentration of product for the remaining mosquitoes (0 and 3.45% at the 0.1% concentration, 75.84 and 8.4 at 0.5%, 80.24 and 19.41% at 1%, for *C. nardus* and *O. americanum* EOs, respectively) (Table 1).

Class 3 contained *E. camaldulensis*, *L. multiflora* EOs, and permethrin which were less irritant at the low concentration (escape rates less than 45% at 0.1% concentration) but activities of *E. camaldulensis* EO and permethrin varied with respect to the concentration of product (escape rates between 52.3 and 69.72%, 58.33 and 78.84% at 0.5, 1% concentrations, respectively). The EO of *L. multiflora* produced higher KD effects that increased with concentration (Table 1).

On the *An. gambiae* VK field strain, class 1 had grouped *C. citratus* EO and DEET which had been irritant at the low concentration with efficiency that increased with respect to the concentration.

Class 2 included *C. nardus*, *L. multiflora*, and *O. americanum* EOs which exhibited decreased activities from low to high concentration. At the same time, *C. nardus* EO produced KD effects that increased with concentration (KD rate = 29.46% at 0.1%, 32.33% at 0.5% and 37.62% at 1%). On the other hand, *L. multiflora* EO produced high KD effects (Table 1).

Class 3 consisted of *E. camaldulensis* EO and permethrin which are not irritant at the low concentration but become efficient when the concentration increases.

In Ae. aegypti **populations**

For the susceptible strain of *Ae. aegypti* Bora Bora, class 1 grouping *C. citratus, E. camaldulensis, O. americanum* EOs, and DEET where their activities increased as the concentration increased except for *O. americanum* EO and DEET.

Class 2, consisting of the EOs of *C. nardus* and *L. multiflora*, grouped products that had activities and decreased as concentration increased. On the other hand, they produced high and increased KD effects when concentration increased (Table 1).

For class 3, permethrin exhibits no irritant effect at low concentrations (0.1% and 0.5%) but becomes irritant at high concentration (1%).

For *Ae. aegypti* strain of Bobo-Dioulasso, class 1 consisting of *C. citratus, E. camaldulensis*, *O. americanum* EOs, DEET, and permethrin which exhibit no irritant effect (escape rates below 50%) at 1 concentration (permethrin and *C. citratus*) or 2 concentrations (*E. camaldulensis*, *O. americanum*, DEET). Class 2 consisted of *C. nardus* EO which was the most irritant at the lowest concentration (72.22% escape rate at 0.1% concentration) but this activity decreased as concentration increased. Conversely, this EO produced increased KD effects when concentrations increased.

Class 3 consisted of *L. multiflora* EO which was more irritant at higher concentrations (escape rates of 52.79 and 58.79% at 0.5 and 1% concentrations, respectively); its activity increased as the concentration increased. Also, this EO produced KD effects that increased with concentration (Table 1).

Discussion

The control of vector-borne diseases constitute an approach that involves personal protection against mosquito bites (Debboun M. and Strckman D. 2012). This approach used commercial repellents that are mainly based on DEET (N, N-diethyl-m-methyl benzamide) (Badolo et al. 2004; Calafat et al. 2012). However, DEET can have harmful side effects (Briassoulis 2001; Clem et al. 1993) and seems to be toxic effects. It is urgent to investigate on other alternative repellents or irritant ingredients specifically the EOs that have possessed the repellent, irritant, and toxic properties. Here, the irritant properties of *Cymbopogon citratus*, *Cymbopogon nardus*, *Eucalyptus camaldulensis, Lippia multiflora*, and *Ocimum americanum* EOs were measured, using the system of Grieco et al. (2005), on *Anopheles* and *Aedes* populations from Bobo-Dioulasso and “Vallée du Kou”, respectively, where pyrethroid resistance was widespread.

Our current study showed that mosquitoes exposed to the five EOs and 2 chemicals as DEET and permethrin showed variable behavioral responses, depending on the type and strain of mosquito, the kind of treatment, and the treatment concentration. Exposure to the five EOs showed varying degrees of contact irritant-repellent activity of these EOs. The irritant effect of a product might be due to its action through tarsi on the nervous system (White 2007). Some individual compounds of essential oils are detected and avoided by mosquitoes through their antennae. Still, the physiological influence of essential oils leading to repellency remains largely unknown (Dekker et al. 2011; Ramirez et al. 2012).

Indeed, *L. multiflora* EO was found to be an irritant on both strains of *An. gambiae* and the susceptible "Bora Bora" strain of *Ae. aegypti*. However, previous studies had shown repellent or irritant properties of β-caryophyllene and p-cymene (Nararak et al. 2019; Ndirangu et al. 2020; Azeem et al. 2019), 2 major compounds of the *L. multiflora* EO tested. This effect of the irritant activity of *L. multiflora* is in agreement with its high "knock-down" effect (90, 88.24, and 87.88% KD at 1% concentration for Kisumu, VK and Bora Bora, respectively) that had prevented mosquitoes to escape. In the same way, in other recent studies, this EO had given high KD effects on the same populations of *Ae. aegypti* and *An. gambiae* (Balboné et al. 2022b, 2022c). In addition to their toxic effect shown recently by Balboné et al. (2022b, 2022c), *L. multiflora* exerted an irritant effect on *Anopheles* and *Aedes* populations. That is useful in terms of its use in vector control strategies.

In most cases, EO of *C. citratus* where activities close to DEET, had a significant irritant effect at the low concentration on the 2 strains of *An. gambiae*, whereas the susceptible strain of *Ae. aegypti* Bora Bora seems to be efficient at high concentrations. Our results corroborate those of Deletré et al. (2013) who showed also irritant effects of *C. citratus* EO on *An. gambiae* susceptible strain. According to these authors, the irritant effect was due to the presence of geranial, neral) (75%) and the activity of this EO increased as the concentration increased. The repellent-irritant effect obtained with *C. citratus* and also *C. nardus* EOs would be facilitated by the large number of non-paralyzed mosquitoes, which are known to produce weak KD effects on *Ae. aegypti* and *An. gambiae* (Balboné et al. 2022a, 2022b). This EO tested recently for its toxicity gave a mortality rate of 0 to 8% (Balboné et al. 2022a, 2022b). Overall, *O. americanum* another EO which is less toxic (Balboné et al. 2022a, 2022b) displayed a significant contact irritant effect on susceptible strains of *Ae. aegypti* and *An. gambiae*, Bora Bora and Kisumu. The relatively high contact irritant bioactivity of *O. americanum* EO could be attributed to its major compounds 1.8 cineole and camphor. Previous studies reported that 1.8 cineole and camphor possessed repellent effects against insects (*Blattella germanica* and *Sitophilus zeamais*, respectively) (Cansian et al. 2015; Chooluck et al. 2019). Moreover, this EO had weak KD effects at high concentration while some authors in their works showed that *O. americanum* EO produced higher KD effects at high concentrations on the susceptible strains, Bora Bora and Kisumu than on the Bobo-Dioulasso and VK field strains of *Ae. aegypti* and *An. gambiae*, respectively (Balboné et al. 2022b, 2022c). On the other hand, studies conducted in Guinea Bissau, West Africa, had reported that *O. americanum* EO had also a repellent effect on Anopheles mosquitoes (Pålsson & Jaenson, 1999).

The two positive controls, DEET and permethrin, were also found to be irritant. Permethrin was a moderately irritant effect on both strains of *An. gambiae* (Kisumu and VK) and weakly irritant to both *Ae. aegypti* (Bora Bora and Bobo-Dioulasso) strains at all concentrations compared to the EOs of *C. nardus*, *L. multiflora* and *O. americanum*. However, it had low KD effects on all strains of both kind of mosquitoes tested, in contrast to the EOs of *L. multiflora*, *C. nardus*, and *C. citratus*, which showed low contact irritancy effects at the highest concentration but high KD effects on the other mosquitoes. Our results are similar to those of Deletre et al. (2013) who showed that permethrin had a weak contact irritant activity on resistant *An. gambiae* populations at high doses. The low irritant effect of permethrin is thought to be due to the increasing resistance of mosquitoes to chemical insecticides and raise the question of its use in Insecticide treated net (ITN). However, previous studies reported that permethrin could still be applied as a repellent against mosquito bites in many places even though pyrethroid resistance was widespread (Bowman et al. 2018) due to the fact that it constitutes a physical barrier. However, it is noteworthy to use permethrin as an irritant at least at concentration (0,5%).

As far as DEET is concerned, it was irritant to all *An. gambiae* strains and caused less KD effect. These results are in agreement with those obtained by Deletre et al. (2013) who showed that DEET was irritant at high concentrations for all *An. gambiae* populations tested. However, on the *Ae. aegypti* strain from Bobo-Dioulasso, DEET was less irritant than all the tested EOs and did not induce KD. These results are not in agreement with those found by Licciardi et al. (2006) who showed that DEET displayed a significant irritant effect and caused the KD effect at high concentrations. This could be explained by the fact that our concentrations were lower than those used by Licciardi et al. (2006). Furthermore, our results show that DEET was more irritant on the *An. gambiae* strains than on the *Ae. aegypti* strains tested whereas previous studies had shown the opposite data (Travis 1947; Rutledge et al. 1983; Curtis et al. 1987; Badolo et al. 2004). This contradiction could be due to the different experimental protocols used. Indeed, in these different studies the tests were carried out with an attractive bait, whereas in our case, the mosquitoes were in an enclosure containing only the tested product.

Conclusion

All the essential oils tested in this study showed irritant effects. On *An. gambiae*, the contact irritant effects of *Cymbopogon citratus*, *Cymbopogon nardus*, and *Eucalyptus camaldulensis* essential oils were very close to those of DEET, the reference repellent. On *Ae. aegypti*, all our EOs had high irritant effects and some of them are very close to DEET. The EOs tested in the present study could be potential mosquito repellent-irritant as alternatives to synthetic contact irritant repellents as pyrethroids and DEET.

Declarations

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Tables

Table 1: Knockdown rates (KD) of 3–7-day old fasting female mosquitoes that remained in the acclimation tubes at end of testing.

		Anopheles gambiae		*Aedes aegypti*	
		"Kisumu"	"VK"	Bora Bora	Bobo-Dioulasso
Treatments	**Dose %**	KD Remained	KD Remained	KD Remained	KD Remained
Cymbopogon	0.1	1.67	0	3.03	0
citratus	0.5	5	0	0	0
	1.	76.93	6.25	16.67	0
Cymbopogon	0.1	0	29.46	18.43	3.33
nardus	0.5	75.84	32.33	76.94	20.1
	1.	80.24	37.62	87.75	61.55
Eucalyptus	0.1	0	0	0	0
camaldulensis	0.5	0	0	0	0
	1.	0	0	0	0
Lippia	0.1	25	5.37	3.14	0
multiflora	0.5	32.85	49.61	34.81	15.74
	1.	49.58	68.43	71.73	44.09
Ocimum	0.1	3.45	0	0	0
americanum	0.5	8.4	0.33	2.5	0
	1.	19.41	3.03	3.33	0
DEET	0.1	3.18	0	0	0
	0.5	5	0	4.85	0
	1.	10	18.98	41.06	0
Permethrin	0.1	0	0	0	0
	0.5	8.33	0	3.33	0
	1.	26.67	5.33	29.16	0

KD: Knock-down; %: percentage

Figures

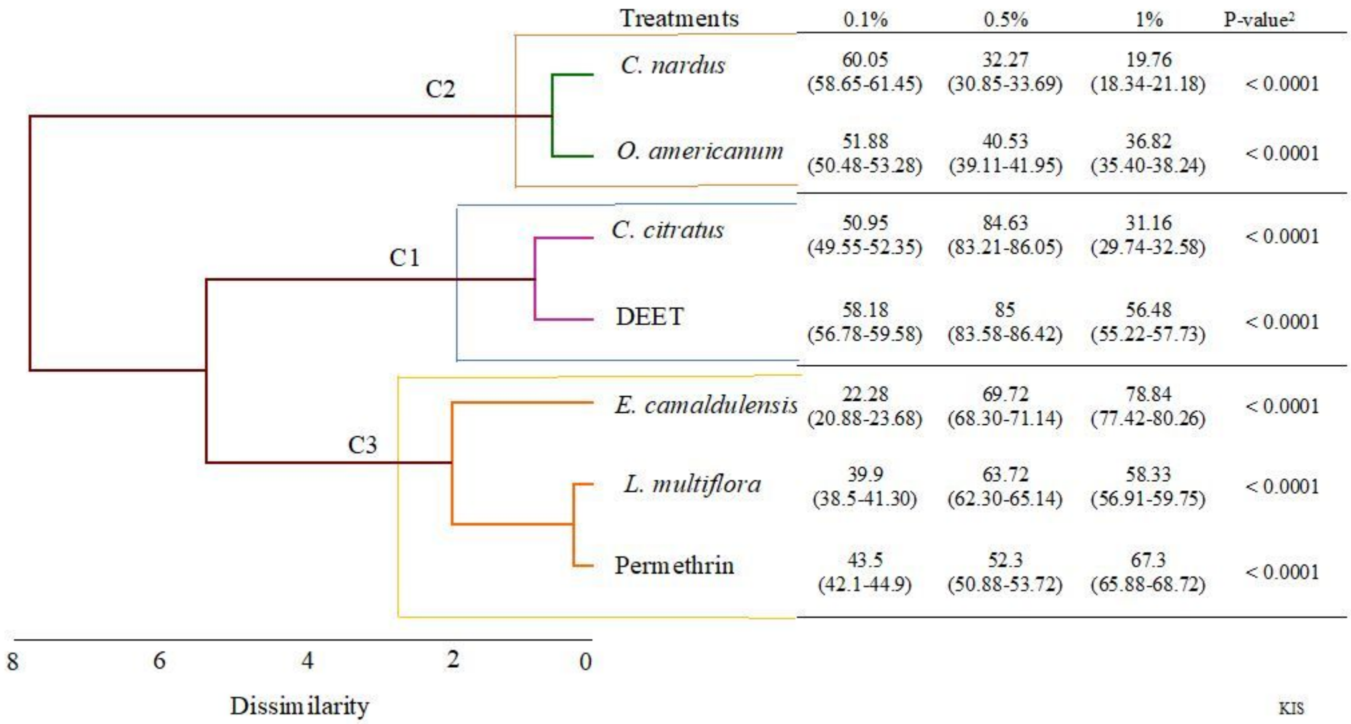

Figure 1

Escape rates of females of the susceptible strain of *Anopheles gambiae* "Kisumu" to the 5 EOs, DEET, and permethrin and, the dendrogram representing the behavioral reactions obtained by the hierarchical clustering of these insecticides according to their contact irritant effects

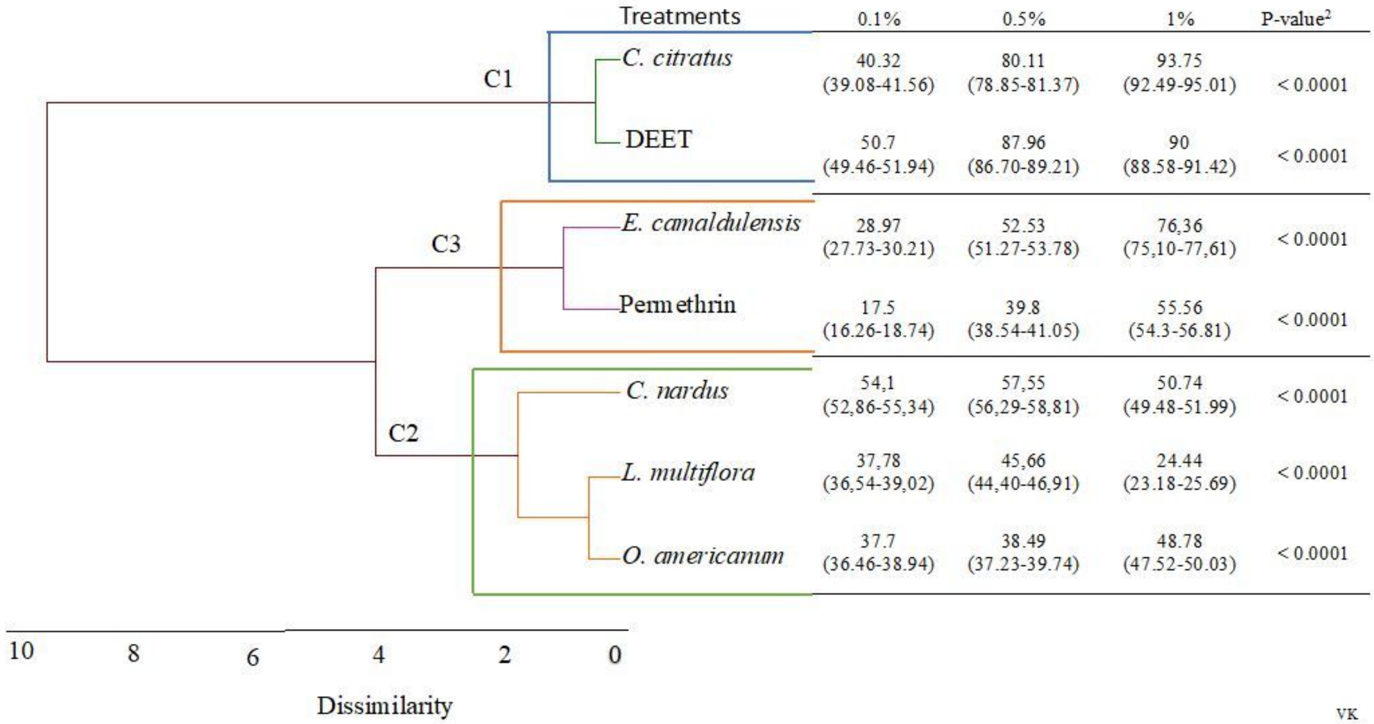

Figure 2

Escape rates of females of the field strain of *Anopheles gambiae* "VK" to the 5 EOs, DEET,and permethrin, and, the dendrogram representing the behavioral reactions obtained by the hierarchical clustering of these insecticides according to their contact irritant effects

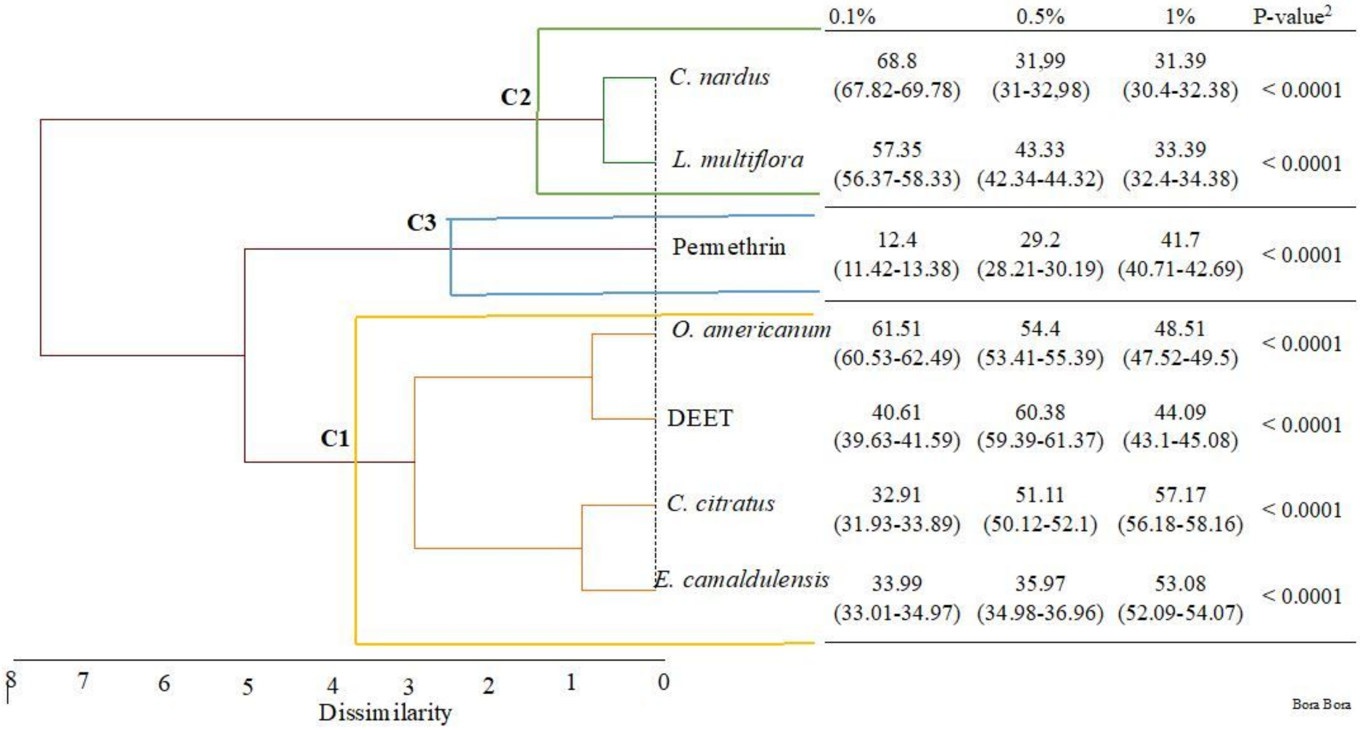

Figure 3

Escape rates of females of the susceptible strain of *Aedes aegypti* "Bora Bora" to the 5 EOs, DEET,and permethrin and, the dendrogram representing the behavioral reactions obtained by the hierarchical clustering of these insecticides according to their contact irritant effects.

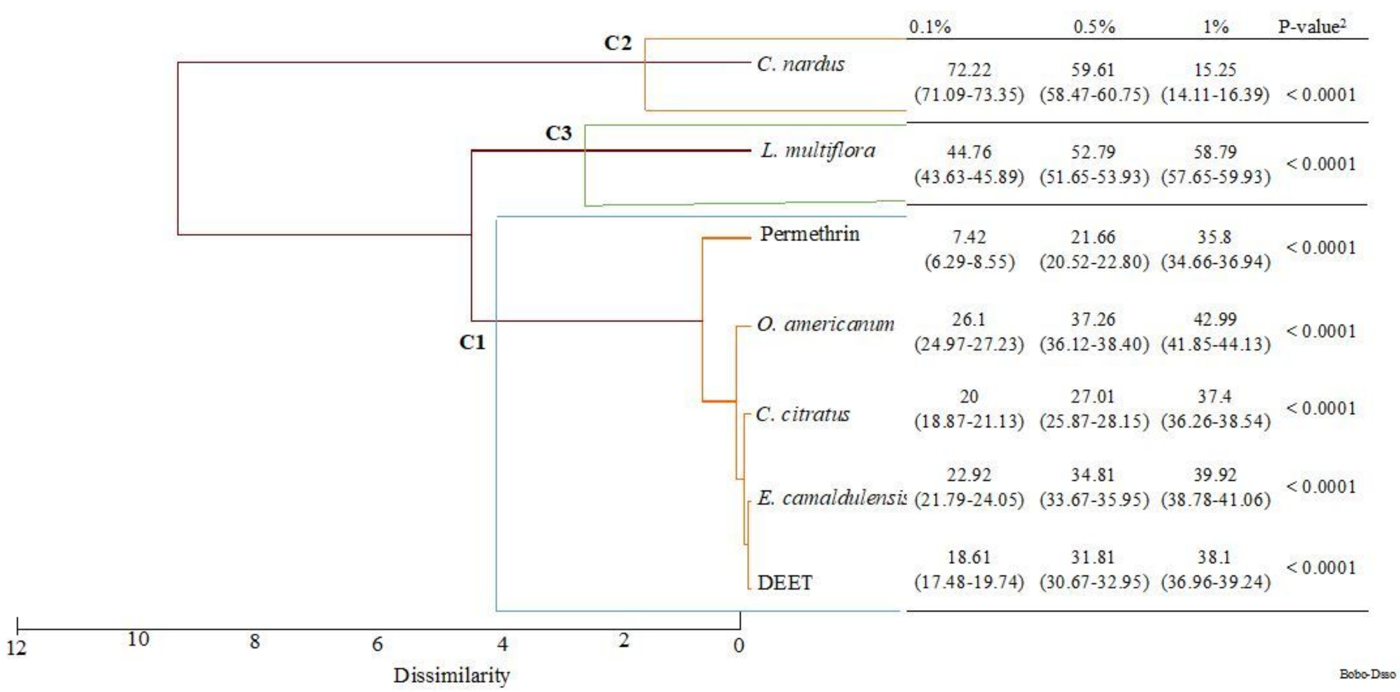

Figure 4

Escape rates of females of the field strain of *Aedes aegypti*"Bobo-Dioulasso" to the 5 EOs, DEET,and permethrin and, the dendrogram representing the behavioral reactions obtained by the hierarchical clustering of these insecticides according to their contact irritant effects.