Alternatives to chemical resistance: Combinations of *Cymbopogon nardus* and *Ocimum americanum* essential oils improve the bioefficiency control against the adults populations of *Aedes aegypti* L. (Diptera: Culicidae).

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Research Article

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Abstract

Dengue vector control strategies are mostly based on chemicals use against *Aedes aegypti* populations. The current study aimed at investigating the insecticidal effects of essential oils obtained from five plant species, *Cymbopogon citratus, Cymbopogon nardus, Eucalyptus camaldulensis, Lippia multiflora* and *Ocimum americanum*, and combinations of *Cymbopogon nardus* and *Ocimum americanum* on *Aedes aegypti* populations from Bobo-Dioulasso. For this purpose, adults of the susceptible and field strains of *Aedes aegypti* were tested in WHO tubes with EO alone and binary combinations of *O. americanum* (OA) and *C. nardus* (CN) (scored from C1 to C9). The extraction of the essential oils was done by hydrodistillation and their components were determined by GC/MS. Among the 5 EOs tested, *L. multiflora* essential oil was the most efficient, with KDT_{50} values below 60 minutes on all *Aedes aegypti* strains tested, and also with a rate of mortality up to 100 and 85% for Bora Bora and Bobo-Dioulasso strains, respectively. This efficacy may be due to its major compounds which are with major compounds as ß-caryophyllene, p-cymene, thymol acetate and 1.8 cineol. Interestingly, on all strains, C8 combination showed a synergistic effect while C2 showed an additive effect. These combinations exhibit a rate of mortality varying from 80 to 100%. Their toxicity would be due to the major compounds and the putative combined effects of some major and minor compounds. More importly, *L. multiflora* EO and combinations of *C. nardus* and *O. americanum* EO, may be used as alternatives against pyrethroid resistants of *Ae. aegypti*.

Introduction

Arboviral diseases have been recognized as a global threat during the last twenty (20) years (Mayer et al. 2016). According to WHO (2021), approximately 3.9 billion people worldwide are at risk of arboviral diseases. These diseases are caused by viruses (arboviruses) usually transmitted, under natural conditions, from vertebrate to vertebrate, by hematophagous insect vector (Aubry 2020) such as *Aedes aegypti* mosquitoes. Zika, chikungunya, yellow fever and dengue are arboviroses whose main vector is *Ae. aegypti* (Souza-neto et al. 2019), (Cuervo-Parra et al. 2016). According to WHO (2020), among the arboviroses, dengue is the most prevalent in the subtropics and tropics areas. Thus, it is a major international public health problem (Guzman et al. 2010). In recent years, it has been detected in several African cities including Ouagadougou and Bobo-Dioulasso, the major cities of Burkina Faso (Sessions et al. 2013), (Ministère de la Santé 2017).

Vector control remains the most effective measure to control the transmission of this arboviral diseases (Bhat and Chavan 2015). The use of insecticides is the main strategy for controlling *Ae. aegypti* (WHO 2012) when a dengue epidemic is detected in a city (Achee et al. 2015). Long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) remain the main vector control methods (WHO 2013).

However, in many countries, resistance to insecticides has been observed in natural populations of disease vectors and this resistance is increasing. Indeed, previous studies in Burkina Faso reported multiple resistance to chemical insecticides commonly used against *Ae. Aegypti* populations (Sombié et al. 2019), particularly in the *Ae. aegypti* population from Bobo-Dioulasso (Ouattara et al. 2019). Recently, Namountougou et al. (2020) highlighted this resistance in *Ae. aegypti* populations in Ouagadougou and Bobo-Dioulasso according to metabolic activities such as non-specific esterase, cytochrome P450 and Glutathione-S-Transferase activities.

To manage concern related to increasing chemical resistance, which has become an obstacle to fight vector-borne diseases in general and dengue specifically, the use of natural products derived from plants with proven insecticidal efficiency is strongly recommended as an alternative. Among the many natural products, essential oils (EOs) and their constituents have received considerable attention in the search for new pesticides exhibiting insecticidal properties (Tripathi et al. 2009). Plant products are used worldwide to protect people against blood-sucking arthropods and many studies have reported the adulticidal effects of essential oils (Zoubiri and Baaliouamer 2014). These natural compounds are biodegradable in nature, environmentally safe (Regnault-roger et al. 2012) and generally exhibit low toxicity to mammals (Isman 2000). Moreover, traditional medicine is largely based on plants (herbs or shrubs) and is available at low cost in most tropical areas (Boer et al. 2010).

EOs have several valuable properties. Firstly, they easily penetrate the cuticle of insects, which increases their bioavailability (Guessan et al. 2007). This property reduces mosquito knockdown time. Regarding the weak toxicity of most EOs against vectors, strategies based on their combination may improve their efficiency. Recent studies on *Aedes aegypti* populations from

Ouagadougou (Burkina Faso) showed low toxicity of 3 of the 5 EOs tested in the present study, namely *Ocimum americanum*, *Cymbopogon nardus*, *Eucalyptus camaldulensis* (Yaméogo et al. 2021). According to Chansang et al. (2018), combination of EO-Permethrin shown a synergist effect on Aedes populations from Thailand.

To the best of our knowledge, even if studies have been carried out on the toxicity of EOs in Burkina Faso, studies on their combinations on *Ae. aegypti* populations are limited. In this study, our objective was to evaluate the insecticidal properties of EOs extracted from 5 local plants, namely *L. multiflora*, *C. nardus*, *O. americanum*, *C. citratus* and *E. camaldulensis*, and binary combinations of 2 of them, namely *O. americanum* and *C. nardus*, against adults of *Ae. aegypti* populations from western Burkina Faso.

Methods

Essential oils extraction

The first step consisted in collecting the aerial parts of the following species: *L. multiflora*, *C. nardus*, *C. citratus*, *E. camaldulensis*, and *O. americanum*. The collection was conducted at the botanic garden of the Institut de Recherche en Sciences Appliquées et Technologies (IRSAT), Ouagadougou. The extraction of EOs was achieved through hydrodistillation using Clevenger apparatus. A dark glass bottle was used for storage at a temperature of 4°C, according to the works done by Drabo et al. (2017). Indeed, the combinations of *C. nardus* and *O. americanum* EOs tests were carried out, after having done the bioassays with the individual EOs, at the "Institut de Recherche en Sciences de la Santé/Direction Régionale de l'Ouest" (IRSS/DRO).

Analysis by means of GC (Gas Chromatography) with FID (Flame Ionization Detector)

Essential oils obtained from the leaves of *L. multiflora, C. citratus, C. nardus, E. camaldulensis and O. americanum* were analysed by GC-FID using an Agilent 6890N GC instrument which is FID equipped, along with a DB-5 narrow bore column. Experimental procedures are detailed in Balbone et al. (2022).

Gas chromatography/mass spectrometry (GC/MS) Analysis

For the GC/MS analysis, a GC HP 6890 connected to an MSD HP 5972 (Hewlett Packard, Palo Alto, CA) with a Zebron ZB-5MS capillary column (30 m long, ID 0.25 mm and film thickness 0.25 mm; Agilent) was used. Experimental procedures are detailed in Balbone et al. (2022).

Collection and rearing of Mosquitoes

The collection of pupae and larvae of the *Ae. aegypti* field strain was carried out from June to October 2021, in Bobo-Dioulasso, West Burkina Faso. Bobo-Dioulasso (11° 10' 37.7" N, 4° 17' 52.4" W), the economic capital of Burkina Faso, is located in the western part of the country. The pupae and larvae were reared in the insectarium located in the "Institut de Recherches en Sciences de la Santé, Direction Régionale de l'Ouest" (IRSS/DRO) in Bobo-Dioulasso. Larvae were fed with tetraMin Baby Fish Food (Tetrawerke, Melle, Germany). Adult mosquitoes which emerged from pupae of the field strain were placed in cages and fed with a 10% sugar solution. Female mosquitoes of first-generation field strain and second-generation *Ae. aegypti* "Bora Bora" strain maintained at the insectarium, as a reference strain, were used for susceptibility tests.

Adult mosquitoes bioassays

Susceptibility tests were performed with WHO tubes according to standard procedures (WHO 2013). Whattman papers were used for impregnation according to the protocol adopted by N'Guessan et al (2007). For this purpose, four rectangular papers (size 12cm × 15cm) were impregnated with 2 ml of a given concentration of an essential oil/combination of EOs diluted in acetone. For combination tests, ten binary combinations varying from C1 to C9 were prepared. Thus, i) CN 10%: OA 90%; ii) CN 20%: OA 80%; iii) CN 30%: OA 70%; iv) CN 40%: OA 60%; v) CN 50% : OA 50%; vi) CN 60% : OA 40%; vii) CN 70% : OA 30%; viii) CN 80% : OA 20% and ix) CN 90% : OA 10% corresponding to C1, C2, C3, C4, C5, C6, C7, C8 and C9, led us to carry out the combination tests.

Preliminary bioassay tests were used to select a range of EO concentrations for the current tests. The concentrations chosen were: 0.1, 0.5 and 1% for each EO or combination of EOs. Acetone was used as a negative control and permethrin 0.75% was used as a positive control. The test conditions were: temperature 25°C (± 2°C) and humidity 70–80%. Diagnostic doses were derived from the susceptible *Ae. aegypti* "Bora Bora" strain.

Data analysis

The data obtained were analyzed using the statistical software XLSTAT version 2015.1.01. By probity analysis, the knock-down times (KDT_{50} and KDT_{95}), lethal concentrations (LC_{50} and LC_{99}) and 95% confidence limits (95% CL) were calculated to compare the toxicity of the EOs and combinations against the adult mosquitoes tested. The LC_{50}, LC_{99} and mortality values were significantly different between the EOs and combinations ($p < 0.05$) if their confidence intervals did not overlap. Corrections were not necessary as no mortality was found in the negative controls. According to OMS (2017), the diagnostic concentration corresponds to Twice the LC_{99} on susceptible strain.

Calculation of the Fractional Inhibitory Concentration indices

Determination of EO interactions in the combinations was done using fractional inhibitory concentration indices or FIC indices. The calculation and interpretation procedures have been described in Schelz et al. (2006), Bassolé and Juliani (2012), Balbone et al. (2022).

Results

Chemical characterization of the essential oils

Data on the chemical analysis of the five EO samples are given in Table 1. *C. citratus* EO was composed of oxygenated monoterpenes (99.9%) represented by 44.7% for neral and 55.2% for geranial. As for *C. nardus* EO*,* it was characterized by the presence of six oxygenated monoterpenes, dominated in majority by citronellal, geraniol and β-Elemene (representing 41.7%, 20.8%, 11%, respectively). Sixteen compounds were found in *E. camaldulensis* EO and 1.8-cineole (59.55%) was the major constituent. In total, sixteen compounds dominated by monoterpenes (69.51%) were identified in the *L. multiflora* EO, among which β-caryophyllene, p-cymene, thymol acetate and 1,8-cineole (representing 20.1%, 14.6%, 12.0%, 11.6%, respectively) were the major constituents. In EO of *O. americanum,* twenty-six (26) compounds dominated by monoterpenes were identified. Major compounds were 1.8 cineol (31.22%) and camphor (12.73%).

Knock-down times and mortality with essential oils

Table 2 summarizes the knock-down times (KDT) with 95% confidence limits (CL95%) and rate of mortality obtained on the two strains of *Ae. Aegypti* tested with EOs. On the susceptible strain Bora Bora, the KDTs were the lowest and mortality rates were the highest than those obtained on the Bobo-Dioulasso strain.

On *Aedes aegypti* strain of Bobo-Dioulasso, the lowest values were obtained with *L. multiflora* EO (KDT_{50} and KDT_{95} = 113.5 and 182.8 min at 0.1%, 43 and 75 min at 0.5%, 20.5 and 32.8 min at 1%, respectively), followed by *C. nardus*, *O. americanum* EOs. The highest KDT values were obtained with *E. camaldulensis* EO (Table 2).

At the highest concentration (1%), there were no significant differences between the KDTs obtained with the EOs on the two *Ae. Aegypti* strains tested, excepted those of *C. nardus* and *L. multiflora*.

On the susceptible strain Bora Bora, the KDT_{50} and KDT_{95} values of *L. multiflora* and *C. nardus* EOs were -3.9 min; 7.8 min and 24; 33.7 min at 1% concentration, respectively.

The tables 2 and 4 showed the rate of mortality, LC_{50} and LC_{99} varying according to the EOs, their concentrations for each strain of *Ae. Aegypti* tested. *L. multiflora* EO was the most toxic whatever the strains with the highest rate of mortality and lowest lethal concentrations (LC_{50} and LC_{99}) (mortality = 85% at 1% concentrations; LC_{50} = 0.79% and LC_{99} = 1.25 for Bobo-Dioulasso strain and rate of mortality of 100% at 1% concentration, LC_{50} = 0.57% and LC_{99} = 1.03 for Bora Bora strain). At 1% concentration, *C. nardus* EO exhibits a rate of mortality of 20.34% and 12.75% on the Bora Bora and Bobo-Dioulasso strains, respectively. *Eucalyptus* *camaldulensis* EO was the less toxic with lowest rate of mortality on both strains (7.14 and 0. 7% at 1% concentration on Bora Bora and Bobo-Dioulasso strains, respectively). On both *Aedes aegypti* strains, *L. multiflora* EO shown rates of mortality that were significantly higher than those found with permethrin 0.75%.

Diagnostic concentrations

Table 4 summarizes the LC_{50}, LC_{99} and diagnostic concentrations of EOs and EO combinations. On both strains of *Ae. aegypti,* the LC_{50} values with combinations C8 (CN 80%; OA 20%), C2 (CN 20%; OA 80%) and C9 (CN 90%; OA 10%) were less than 1% while those of C4 (CN 40%; OA 60%) and C6 (CN 60%; OA 40%) were less than 2%.

The lowest diagnostic concentrations were obtained with *L. multiflora* EO and combinations C8 and C2, with values of 2.06, 2.16 and 2.56% respectively (Table 4). The highest diagnostic concentrations were obtained with C5, followed by *E. camaldulensis* and C1 (CN 10%; OA 90%). Except for C5 (CN 50%; OA 50%) and C1, all combinations had lower diagnostic concentrations than those of *C. nardus* and *O. americanum* EOs (Table 4).

Knock-down times and rates of mortality with combinations of *Cymbopogon nardus* and *Ocimum americanum*

The knock-down times (KDT) values and rates of mortality obtained with the combinations of *C. nardus* and *O. americanum* EOs on both strain of *Aedes aegypti* varied according to the proportions of EOs in the combinations and the concentrations used (Table 3).

On both *Ae. aegypti* strains, the lowest KDT values were obtained through the combination C8 (CN 80%: OA 20%, i.e., 80 and 20% of *C. nardus* and *O. americanum*, respectively), followed by C2 and the highest values were obtained with C3 (CN 30%: OA 70%) and C1. Indeed, the KDT_{50} and KDT_{95} values with C8 were 48.9 and 77 min at 1% on Bora Bora strain, 60.6 and 79.8 min on Bobo-Dioulasso strain, respectively. Overall, all combinations showed low KDT values. Indeed, on both strain of *Aedes aegypti*, the KDT_{95} values were lower than 60 min at 1%, except for combinations C3 and C1 which showed KDT_{50} values of 77.5 and 110.7 min, KDT_{95} of 186 and 172.4 min at the 1%, respectively. On both strains of *Ae. aegypti*, the KDT values obtained with the combinations C8, C2 and C9 were significantly lower than those of the EO tested solely at all concentrations.

The highest rate of mortality at 1% concentration were 100, 86.67% on the Bora Bora strain and 95.09, 81.99% on the Bobo-Dioulasso strain. These rates were obtained with the C8 and C2 combinations, respectively. As for Combination C9, a rate of mortality of 51.76% at 1% concentration was found. The rates of mortality found with the C2, C8 and C9 combinations were significantly higher than those of the EOs tested separately. On the contrary, the rates of mortality of the other combinations were significantly higher than those of the *O. americanum* EO alone. The lower rate of mortality was obtained with the C1 combination (2.66 and 1.6% at the 1% concentration on the Bora Bora and Bobo-Dioulasso strains, respectively).

Effects of combinations against *Aedes aegypti*

Tables 5 and 6 summarize the effects of the interactions of the two EOs in the combinations (Tables 5 and 6). Most efficient combinations regarding knock-down times and mortality rates were those that gave synergistic and additive effects. The synergistic effect was obtained on both strains with the combination C8 and the additive effect was found with the combinations C2 and C9 (Tables 5 and 6).

Discussion

Vector control strategies have mainly consisted of the use of chemicals against mosquitoes. The frequent use of chemicals has raised many concerns in the environment and human public health (Cuervo-Parra et al. 2016) and caused increasing resistance in most mosquito populations (Mahanta et al. 2019) and particulary *Ae. aegypti* (Namountougou et al. 2020, Badolo et al. 2019). Regarding this situation, plants extracts seem to be an alternative because they contain large number of bioactive, effective, biodegradable compounds and have been used currently for mosquito control (Boonyuan et al. 2014, Yaméogo et al. 2021). From the present knowledge, these are the first time that works on toxicity of combinations of whole EOs on *Ae. aegypti* populations were investigated.

Here, our current study showed the variable adulticidal effects of EOs of *C. nardus, C. citratus, O. americanum, L. multiflora* and *E. camadulensis* on susceptible Bora Bora and resistant *Aedes aegypti* strain collected in Bobo-Dioulasso. These results partially confirm the work of Yameogo et al. (2021) who showed the adulticidal effects of some identical EOs tested on *Ae. aegypti* populations collected in Ouagadougou. They also confirm the works done by Drabo et al. (2017) who had shown the adulticidal properties of *C. citratus* and *O. americanum* on *Bemisia tabaci* populations confirming their broad-spectrum efficiency against most of insects.

Interestingly, the EO of *L. multiflora* was the most toxic on both strains of *Ae. aegypti*. This toxicity of *L. multiflora* EO could be explained by the insecticidal effect of the most dominant compounds which are thymol acetate, p-cymene and ß-caryophyllene. Indeed, previous studies reported that ß-caryophyllene exhibits insecticidal properties (Almadiy 2020) and toxicity of *Eucalyptus tereticornis* EO could be explained by the presence of the other component as p-cymene (Bossou et al. 2013) found as a major component in *L. multiflora*. Other previous studies reported that the insecticidal properties of *O. americanum* EO were due to 1.8-cineole, camphor and α-pinene (Koul et al. 2008). These compounds were the same of those found in *O. americanum* EO tested here and for which we obtained the weak efficacy. According to Ntonga et al. (2017), 1.8-cineole is reported to contribute strongly to the toxicity of *Callistemon citrinus* oil against adult females of *Anopheles coluzzii.* On the contrary, *E. camaldulensis* EO, rich in 1,8-cineole was the least toxic against adult females of *Ae. aegypti*. This low toxicity could be due to the absence of sabinene among the main compounds of *E. camaldulensis* EO. Indeed, Liu et al (2020) reported that there was an additive effect between 1.8-cineole and sabinene on *Sitophilus oryzae*.

If the pre-exposure of mosquitoes to PBO, can restore totally or partially the susceptibility to pyrethroids (Hien et al. 2021), there is no doubt that the toxicity of EOs could be improved by binary combinations of certain EOs.

In the current study, this improvement was outlined through the KDT, LC_{50} values and rate of mortality obtained with certain combinations which were higher than those of the EOs tested alone. It is suggested that combinations reduce the time required to paralyze mosquitoes.

Interestingly, the combination of C8 (CN 80%: OA 20%) and C2 (CN 20%: OA 80%) improved the toxicity on the *Aedes* populations. This would be due to the simultaneous toxic effect of the compounds in the combined EOs. It could be thought that *C. nardus* EO needed a certain amount of the major compounds of *O. americanum* EO. Conversely, *O. americanum* EO needed some constituents of *C. nardus* EO to perform. Studies had shown that the addition of a little citronellal enhanced the effect of the citral-myrcene mixture against *Ae. aegypti*. Nevertheless, mosquito host-seeking behavior was not significantly reduced by citronellal alone (Hao et al. 2008). Similarly, lethal toxicity of the main components of *Ocimum kilimandscharicum* (such as camphor, 1.8-cineole and ß-caryophyllene) EOs had been reported against two insect pests (*Rhyzopertha dominica* and *Sitophilus zeamais*) (Bekele and Hassanali 2001). According to these authors, the major compounds found in *O. kilimandscharicum* EO had high toxic effect against tested insects.

Synergistic effect obtained by some of our combinations could also be explained by the simultaneous action of the major compounds. Indeed, combinations of 1.8-cineole and citronellal as well as several minor compounds and citronellal gave synergistic effects on *Spodoptera littoralis* larvae (Pavela 2014). According to Hildgaard et al (2012), each compound of an EO had a specific mode of action but these compounds could interact with compounds of another EO in a combination to give other modes of action. Thus, the combination of compounds with different modes of action could increase the total lethal actions in mixtures (Sarma et al. 2019).

This study opens new perspectives of valorization of EOs. According to the Gnankine and Bassole (2017), microencapsulation technologies may improve the duration of action of EOs in terms of application taking into consideration the fact that the EOs are volatiles.

Conclusion

Our study investigated the adulticidal effects of the essential oils of five plants used traditionally in Burkina Faso, *Lippia multiflora, Cymbopogon nardus, Ocimum Americanum, Cymbopogon citratus* and *Eucalyptus camaldulensis*, and combinations of two of these EOs against adults of *Aedes aegypti*, the mosquito vector of dengue. *L. multiflora* EO was the most toxic on both strains of

Aedes aegypti tested. EOs of *C. nardus* and *O. americanum* were less toxic but their combinations were more toxic, in some proportions, to both strains of *Ae. aegypti*. *L. multiflora* EO and combinations of C8 (CN 80%: OA 20%), C2 (CN 20%: OA 80%) and to a lesser extent C9 (CN 90%: OA 10%) could be promising alternatives in control strategies against *Ae. aegypti*, the dengue vector. As far as we know, this study demonstrates the first time that the binary combinations namely C8 and C2 exhibit high rates of mortality on Bobo-Dioulasso resistant strain to chemicals.

Declarations

Competing interests: The authors declare no competing interests.

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Tables

Table 1 Chemical composition of *Lippia multiflora, Cymbopogon nardus, Ocimum americanum, Cymbopogon citratus,* and *Eucalyptus camaldulensis* essential oils

Compounds	RI	*Cymbopogon citratus* (%)	*Cymbopogon Nardus* (%)	*Eucalyptus Camaldulensis* (%)	*Lippia multiflora* (%)	*Ocimum americanum* (%)
α-Thujene	925	-	-	-	1.6	0.13
α-Pinene	933	-	-	9.17	0.9	6.87
Camphene	945	-	-	0.19	-	2.77
Sabinene	975	-	-	-	-	-
β-pinene	981	-	-	2.49	-	0.65
Myrcene	992	-	-	0.15	-	3.71
2-carene	999	-	-	-	-	2.04
α-Phellandrene	1003	-	-	0.32	3.1	0.39
α- Terpinene	1017	-	-	-	1.41	0.40
p-Cymene	1027	-	-	4.73	**14.6**	0.21
Limonene	1028	-	-	8.76	-	4.66
1,8-cineole	1034	-	-	**59.55**	**11.6**	**31.22**
β-Ocimene	1048	-	-	-	9.4	-
γ-Terpinene	1061	-	-	1.39	-	1.13
Terpinolene	1081	-	-	0.21	-	0.65
Camphor	1124	-	-	-	-	**12.73**
Citronellal	1158	-	**41.7**	-	-	-
Citronellol	1230	-	8.0	-	-	-
Borneol	1156	-	-	0.46	-	0.13
4-Terpineol	1168	-	-	1.14	-	0.92
α-Terpineol	1177	-	-	2.65	-	2.08
Neral	1242	**44.7**	-		-	
Piperitone	1252	-	-	0.09	-	2.44
Geraniol	1253	-	**20.8**	-	-	-
Geranial	1268	**55.2**	-	-	-	-
Bornyl acetate	1269	-	-	-	-	3.96
Thymol	1288	-	-	-	8.3	-
Thymol acetate	1355	-	-	-	**12.0**	-
β-Elemene	1372	-	**11.0**	-	-	-
α−Copaene	1390	-	3.7	-	-	-
β-Caryophyllene	1415	-	-	-	**20.1**	3.55

Trans-α-Bergamotene	1433	-	-	-	-	5.32
α-Humulene	1442	-	-	-	4.8	0.25
allo-Aromadendrene	1446	-	-	1.40	-	-
α -Amorphene	1485	-	-	-	1.4	-
Germacrene D	1495	-	-	-	1.5	0.24
(Z, E)-α-farnesène	1497	-	-	-	-	3.64
B-himachalene	1499	-	-	-	-	-
Hedycaryol	1520	-	7.4	-	-	-
Elemol	1545	-	-	-	2.1	1.6
β-eudesmol	1650	-	-	-	2.5	-
Caryophyllene oxyde	1581	-	-	-	4.5	0.08
Hydrocarbon monoterpenes		-	-	**24.89**	**33.01**	**23.61**
Oxygenated monoterpenes		**99.9**	**77.9**	**66.96**	**36.5**	**53.48**
Hydrocarbon sesquiterpenes		**-**	**14.7**	**1.4**	**25.8**	**13.03**
Oxygenated sesquiterpenes		**-**	**-**	**0.34**	**4.5**	**1.68**
Total		**99.9**	**92.6**	**93.59**	**99.81**	**91.8**

Table 2 KDT_{50}, KDT_{95} and mortalities from essential oils tested on *Aedes aegypti* susceptible (Bora bora) and field (Bobo-Dioulasso) strains

Treatments		*Aedes aegypti "Bora bora"*			*Aedes aegypti "Bobo-Dioulasso"*		
	Doses	KDT_{50} (Min)	KDT_{95} (Min)	Mortality (%)	KDT_{50} (Min)	KDT_{95} (Min)	Mortality (%)
C. citratus	0.1	193.3	291.1	0	221.2	311.2	0
	0.5	192.6	290.2	4.04	228.1	298.5	0
	1	140.6	230.4	8.26	142.5	244.8	0.9
C. nardus	0.1	88.6	134	3.1	179	278	0.98
	0.5	35.6	53.8	9.86	88.7	143.9	5
	1	24	33.7	20.34	35.6	57.2	12.75
E. camaldulensis	0.1	211.3	299.1	0	234.1	343.9	0
	0.5	178.1	285.7	3.3	209.4	297.2	0.1
	1	156.5	267.5	7.14	190.3	287.2	0.7
L. multiflora	0.1	107	178.7	12.35	113.5	182.8	4.1
	0.5	40.9	72	29.33	43	75	16.02
	1	-3.9	7.8	100	20.5	32.8	85
O. americanum	0.1	131.57	194.82	2.08	151.7	264.3	0
	0.5	128.1	188.4	5.56	145.3	247.5	0.2
	1	125.7	210.6	9.14	133.3	249	2.3
Permethrin	0.75	16.6	29.1	98.8	144	188.7	10.1

Table 3 KDT_{50}, KDT_{95} and mortalities of binary combinations of *C. nardus* (CN) and *O. americanum* (OA) tested on *Aedes aegypti* susceptible (Bora bora) and field (Bobo-Dioulasso) strains

Codes	Combination of essential oil	*Aedes aegypti* "Bora bora"				*Aedes aegypti* "Bobo-Dioulasso"		
		Doses	KDT_{50} (Min)	KDT_{95} (Min)	Mortality (%)	KDT_{50} (Min)	KDT_{95} (Min)	Mortality (%)
C1	CN 10% : OA 90%	0.1	117.5	159	0	127.3	187.4	0
		0.5	95	136.3	0.6	118.3	180.7	0
		1	76.5	116.2	2.66	110.7	172.4	1.6
C2	CN 20% : OA 80%	0.1	103.1	169.7	1.23	125.7	173.7	0.4
		0.5	65	113.4	7.23	103.3	163.4	6.90
		1	-4	0.9	86.67	1.2	9.2	81.99
C3	CN 30% : OA 70%	0.1	147.7	228.1	0	175.7	273.7	0
		0.5	98.2	164.8	1.16	118.6	252.7	0.3
		1	28.4	48.5	5.49	77.5	186	4.27
C4	CN 40% : OA 60%	0.1	173.5	281.3	0	190.3	287.2	0.9
		0.5	65.5	107.3	2.47	113.3	184.5	2.19
		1	15.2	33.2	15.96	17	42.2	7.49
C5	CN 50% : OA 50%	0.1	123.4	197.3	4.65	131.7	222.4	2.30
		0.5	76.6	139.4	8.33	102.2	168.8	4.55
		1	17.1	28.2	10.11	27	47.1	7.82
C6	CN 60% : OA 40%	0.1	96.2	161.2	0	151.7	252.3	0
		0.5	24.2	36.7	2.44	106.1	160.1	1.10
		1	10.5	16.7	10.31	17.1	22	9.89
C7	CN 70% : OA 30%	0.1	146	217	0	189.9	286.7	0
		0.5	115.3	184.1	1.1	130.8	199.7	0.31
		1	25.5	37.3	6.02	38.5	57.1	2.20
C8	CN 80% : OA 20%	0.1	48.9	77	3.37	60.6	79.8	3.19
		0.5	11.6	59.2	20.99	14.3	46.5	13.75
		1	-9.8	0.6	100	-3.9	7.8	95.09
C9	CN 90% : OA 10%	0.1	92.3	137.2	2.38	115.4	157.8	1.28
		0.5	13.5	40.5	39.73	20.5	53.8	30.23
		1	7.7	12.3	58.24	8.5	17.1	51.76
Permethrin		0.75	16.6	29.1	98.8	144	188.7	10.1

Table 4 Lethal concentrations at 50 and 99% (LC_{50} and LC_{99}) and diagnostic concentration of all essential oils and combinations of *O. americanum* and *C. nardus* tested with *Aedes aegypti*

Treatments	*Aedes aegypti* "Bora Bora"		*Aedes aegypti* Bobo-Dioulasso		Diagnostic concentration (%)	Diagnostic concentration (mg/ml)	Diagnostic concentration (mg/cm2)
	CL_{50}(%) (CL95)	CL_{99}(%) (CL95)	CL_{50}(%) (CL95)	CL_{99}(%) (CL95)			
C. citratus	2.7 (-)	4,9 (-)	-	-	9.88	98.8	1.10
C. nardus	2.24 (1.6-2.7)	4.3 (2.7-4.9)	2.94 (1.4-3.8)	5,9 (5.7-8.7)	8.6	86	0.95
E. camaldulensis	3.58 (3.3-4.2)	7.81 (-)	-	-	15.62	156.2	1.73
L. multiflora	0.57 (0.5-0.6)	1.03 (0.9-1.2)	0.79 (0.7-0.8)	1,25 (1.1-1.4)	2.06	20.6	0.23
O. americanum	2.7 (-)	4.9 (3.4-5.3)	3.1 (-)	5.3 (-)	9.8	98	1.09
C1 (CN 10% : OA 90%)	3.63 (-)	6.41 (-)	3.9 (-)	6.8 (-)	12.72	127.2	1.41
C2 (CN 20% : OA 80%)	0.77 (0.7-0.8)	1.28 (1.2-1.4)	0.84 (0.8-0.9)	1.297 (1.2-1.4)	2.56	25.6	0.28
C3 (CN 30% : OA 70%)	2.683 (1.46-5)	4,9 (3.2-5.9)	2.6 (-)	4.5 (-)	8.4	84	0.93
C4 (CN 40% : OA 60%)	1.49 (1.2-2.2)	2.66 (2-4.6)	1.98 (1.5-2.3)	3.9 (2.4-4.6)	5.06	50.6	0.56
C5 (CN 50% : OA 50%)	4.02 (-)	9.697 (-)	3.83 (-)	8.3 (-)	19.4	194	2.16
C6 (CN 60% : OA 40%)	1.79 (1.4-3.5)	3.27 (2.3-7.7)	1.68 1.3-3.3	2.92 (2.1-3.1)	6.54	65.4	2.16
C7 (CN 70% : OA 30%)	1.7 (1.3-1.9)	2.94 (1.9-4.4)	3.47 (-)	6.1 (-)	5.88	58.8	0.65
C8 (CN 80% : OA 10%)	0,6	1,08	0,7	1,2	2.16	21.6	0.24

	(0.5-0.6)	(1-1,2)	0,6-0.8	(1.1-1.3)			
C9 (CN 90% : OA 10%)	0.97 (0.8-1.1)	2.29 (1.9-3)	0.99 (0.9-1.3)	2.56 (2-3.5)	4.58	45.8	0.51

Table 5: The effects of interaction of essential oil combinations from *Cymbopogon nardus* (C.n) and *Ocimum americanum* (O.a) on *Aedes aegypti* adults of the susceptible strain (Bora Bora) and the type of interaction (n = 150 adults).

Combination of essential oil (%)	Codes	mortality at LC_{50} (%)	FIC_{Cn}	$FIC_{O.a}$	FIC	Effect
CN 0% : OA 100%	OA	2.7	-	-	-	-
CN 10% : OA 90%	C1	3.61	1.61	1.34	2.95	No effect
CN 20% : OA 80%	C2	0.77	0.34	0.29	0.63	Addition
CN 30% : OA 70%	C3	2.35	1.05	0.87	1.92	No effect
CN 40% : OA 60%	C4	1.48	0.66	0.55	1.21	No effect
CN 50% : OA 50%	C5	3.31	1.48	1.23	2.70	No effect
CN 60% : OA 40%	C6	1.79	0.80	0.66	1.46	No effect
CN 70% : OA 30%	C7	1.78	0.79	0.66	1.45	No effect
CN 80% : OA 20%	C8	0.6	0.27	0.22	0.49	Synergistic
CN 90% : OA 10%	C9	0.97	0.43	0.36	0.79	Additive
CN 100% : OA 0%	CN	2.24	-	-	-	-

$FIC = FIC_A + FIC_B$; Additive: $0,5 \leq FIC \leq 1$; Synergistic: $FIC < 0,5$; Antagonistic: $FIC > 4$; No effect: $1 \leq FIC \leq 4$.

Table 6: The effects of interaction of essential oil combinations from *Cymbopogon nardus* (CN) and *Ocimum americanum* (OA) on *Aedes aegypti* adults of the field strain (Bobo-Dioulasso) and the type of interaction (n = 150 adults)..

Combination of Essential oil (%)	Codes	Mortality at LC_{50} (%)	FIC_{Oa}	FIC_{Cn}	FIC	Effect
CN 0% : OA 100%	OA	3.1	-	-	-	-
CN 10% : OA 90%	C1	3.9	1.25	1.32	2.57	No effect
CN 20% : OA 80%	C2	0.84	0.27	0.28	0.55	Additive
CN 30% : OA 70%	C3	2.6	0.83	0.88	1.71	No effect
CN 40% : OA 60%	C4	2.18	0.7	0.74	1.44	No effect
CN 50% : OA 50%	C5	3.83	1.23	1.3	2.53	No effect
CN 60% : OA 40%	C6	1.68	0.54	0.57	1.11	No effect
CN 70% : OA 30%	C7	3.47	1.11	1.18	2.29	No effect
CN 80% : OA 20%	C8	0.7	0.22	0.24	0.46	Synergistic
CN 90% : OA 10%	C9	1.05	0.34	0.36	0.7	Additive
CN 100% : OA 0%	CN	2.94	-	-	-	-

$FIC = FIC_A + FIC_B$; Additive: $0,5 \leq FIC \leq 1$; Synergistic: $FIC < 0,5$; Antagonistic: $FIC > 4$; No effect: $1 \leq FIC \leq 4$.