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Chemical Composition and Insecticidal Activity of Essential oils of two Aromatic plants from Ivory Coast against *Bemisia tabaci* G. (Hemiptera: Aleyrodidae)

Etienne V. Tia[a], Augustin A. Adima[b], Sébastien L. Niamké[a], Gnago A. Jean[b], Thibaud Martin[c], Paul Lozano[c2] and Chantal Menut[d*]

[a]*Laboratoire de Biotechnologies option Sciences des Aliments, Unité de Formation et de Recherche(UFR) Biosciences, Université de Cocody BP V84 Abidjan Côte d'Ivoire*
[b]*Laboratoire de chimie de l'eau et des Substances Naturelles (LACESNA), Institut National Polytechnique Houphouët Boigny (INP-HB), BP 1093 Yamoussoukro-Côte d'Ivoire*
[c]*Cirad Montpellier, France. 1-UR Hortsy 2-UMR GPEB*
[d]*Institut des Biomolécules Max Mousseron (IBMM)-UMR 5247, ENSCM, 8, rue de l'Ecole Normale 34296 Montpellier Cedex 5, France*

chantal.menut@univ-montp2.fr

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Essential oils of aromatic plants with insecticidal properties are nowadays considered as alternative insecticides to protect cultures from attack by insect pest. The aims of the present work were to evaluate the toxicity of the essential oils vapors of two aromatic plants (*Lippia multiflora* Mold. and *Aframomum latifolium* K. Schum) against *Bemisia tabaci* and to characterize their chemical composition. The highest fumigant toxicity against *B. tabaci* adults was observed with the *L. multiflora* oil: by exposure to 0.4 µL/L air, the lethal time inducing 90% mortality (LT_{90}) was below 2 hours for this essential oil whereas it reached 15 h in the case of the *A. latifolium* oil. Both oils were analyzed by GC-FID and GC-MS on two capillary columns. The oil of *L. multiflora* contained a majority of oxygenated terpenoids mainly represented by the two acyclic components linalool (46.6%) and (*E*)-nerolidol (16.5%); the oil of *A. latifolium* was dominated by hydrocarbonated terpenoids among them β-pinene (51.6%) and β-caryophyllene (12.3%) were the two major components.

Key words: essential oil; chemical composition; vapor toxicity; *Bemisia tabaci*; *Lippia multiflora; Aframomum latifolium*; biocide.

The whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) is a polyphagous insect pest widespread worldwide, particularly in subtropical Africa. Of Indian origin [1], it had a wide range of host feeding on 300 plant species belonging to 63 families [2]. With the development of the highly polyphagous biotype B, *B. tabaci* has become a pest of greenhouse crops in many parts of the world and it is estimated that it currently has a range of about 600 host plant species. This insect causes direct damage by feeding, leading to decline in the crop and decrease in yield, and indirect damage such as honeydew secretion, which allows sooty mould to develop. It is also vector of viruses which cause economically important losses in production. This whitefly has been studied worldwide for its capacity to acquire and spread viruses [3].

For the 90's in West Africa, outbreaks of *B. tabaci* have been regularly observed in cotton and in tomato crops [4]. This pest was particularly difficult to control because of its resistance to many chemical insecticides [5,6] which could be partly due to the presence of an invasive Q1-biotype recently observed in Burkina Faso on cotton and vegetables [7]. It seems that no specific treatment can be used for a long-term protection against this pest and that the combination of a number of control agents must be implemented to get an effective control. Among the current strategies for seeking alternatives to reduce the use of conventional insecticides, eco-chemical control based on plant-insect relationship would be one of the most promising methods. Natural pesticides based on plant-essential oils may represent alternative for crop protection. Essential oils, obtained by steam distillation of plant foliage, and even the foliage itself of certain aromatic plants (notably in the families Myrtaceae and Lamiaceae, but in other plant families as well) have traditionally been used to protect stored grain and legumes, and to repel flying insects in the home. Most of the active compounds of the essential oils are specific to particular insect groups and not to mammals [8]; they have demonstrated contact and fumigant toxicity to a number of economically important insect and mite pests, as well as to plant pathogenic fungi [9].

Lippia multiflora Moldenke (Verbenaceae) syn. *L. adoensis* Moscht is a shrubby aromatic plant, growing up to 1.2 m with whitish flowers on cone-like heads in a

Table 1: Yield and physical characteristics of essential oils of *L. multiflora* and *A. latifolium* from Ivory Coast.

Parameter	*L. multiflora*	*A. latifolium*
Yield (g/100g)	**1.2**	**0.37**
Density (d^{20}_{20})	**0.87**	**0.86**
Refractive index (n_D^{20})	**1.475**	**1.475**

terminal panicle, and nearly 12 mm long. It is widely distributed in West and Central Tropical Africa [10]. In Ivory Coast, it is found in savannah zones. Locally, the plant is named '*Magnrin*' according to the people "Baoulé" of Yamoussoukro. The leaves are recommended in folk medicine to treat hypertension and malaria [11]. Traditionally, *L. multiflora* has been used as a substitute for tea and as a mouth disinfectant [12]. In most cases, the parts used are leaves or aerial parts and flowers. They are commonly prepared as an infusion or decoction and administrated orally [13].

A survey of the available literature shows a great variability of essential oils composition within the genus *Lippia* [14]. Further investigations were preformed in the South America area, supporting this chemical variability [15a-15c] as well as the biological interest of their essential oils [15d,15e] as a larvicidal activity against *Aedes aegypti* [16]. In a recent report on the chemical composition and antibacterial activity of the essential oil of *L. multiflora* from Nigeria [17], the various chemotypes previously identified for this botanical species were reviewed once more: they were characterized by high terpenoids contents, represented by acyclic components (linalool, citral, myrcene, epoxymyrcene, ocimenone, ipsdienone, tagetones, ipsenone, geraniol, β-farnesene, nerolidol), monocyclic components (limonene, γ-terpinene, *p*-cymene, 1,8-cineole, α-terpineol, thymol/thymyl acetate) or bicyclic structures (sabinene, myrtenol or (*E*)-caryophyllene). The oil of *L. multiflora* obtained from Nigeria was characterized by its richness in 1,8-cineole (60.5%), sabinene (16.9%) and α-terpineol (14.1%). In a study of the essential oils obtained from leaves of *L. multiflora* collected in twelve different regions in Ghana, the authors confirm the chemical diversity of this species and propose a classification of their samples on the basis of a cluster analysis, in three chemical groups, with nevertheless the same characteristic components: (*E*)-β-farnesene, (*E*)-β-caryophyllene and β-bisabolene in the "sesquiterpene group", p-cymene, thymol and thymyl acetate in the "aromatic monoterpene group" and 1,8-cineole (42.5-46.9%) in the "cineole group". A single sample could not be classified with the others: the essential oil was characterized by linalool (28.8%), germacrene D (27.9%) and β-caryophyllene (9.5%) [18]. To the best of our knowledge, there are only few previous reports on the essential oil of *L. multiflora* from Ivory Coast.

The two first ones described quite different composition: (*E*)/(*Z*)-tagetones (30.2%/11.3% respectively) and ipsenone (8.9%) were the main components of a sample

Table 2: Chemical composition of essential oils from leaves of *L. multiflora* and *A. latifolium* collected in Ivory Coast.

Compounds	LRI[a]	LRI[b]	*L. multiflora* (%)	*A. latifolium* (%)	Method of identification
α-Thujene	927	-	-	1.0	MS, LRI
α-Pinene	935	1011	0.2	**6.7**	GC, MS, LRI
Camphene	951	1049	-	0.1	GC, MS, LRI
Sabinene	975	1112	0.9	**7.6**	GC, MS, LRI
β-Pinene	982	1104	0.2	**51.6**	GC, MS, LRI
Myrcene	991	1152	0.3	0,6	GC, MS, LRI
α-Phellandrene	1004	1154	1.0	0.1	MS, LRI
α-Terpinene	1017	1166	-	0.2	MS, LRI
p-Cymene	1023	1260	0.2	0.3	GC, MS, LRI
Limonene	1030	1187	-	1.0	GC, MS, LRI
β-Phellandrene	1031	1190	0.1	3.1	MS, LRI
1,8-Cineole	1031	1203	3.2	-	GC, MS, LRI
(*E*)-β-Ocimene	1051	1242	0.6	0.1	GC, MS, LRI
γ-Terpinene	1060	1232	0.1	0.4	MS, LRI
cis-Sabinene hydrate	1068	-	0.1	0.1	MS, LRI
Terpinolene	1091	1267	0.1	0.2	MS, LRI
Linalool	1097	1552	**46.6**	-	GC, MS, LRI
trans-Sabinene hydrate	1098	1468	-	0,1	MS, LRI
trans-Pinocarveol	1125	-	-	0.1	MS, LRI
δ-Terpineol	1170	-	0.1	-	MS, LRI
Terpinen-4-ol	1181	1595	0.1	0.7	GC, MS, LRI
α-Terpineol	1194	1686	0.9	0.2	GC, MS, LRI
Myrtenol	1196	1782	-	0.2	MS, LRI
cis-p-Mentha-1(7),8-dien-2-ol	1228	-	0.6	-	MS, LRI
Linalyl acetate	1254	1550	0.8	-	GC, MS, LRI
Bornyl acetate	1289	1585	-	0.1	GC, MS, LRI
cis-Sabinyl acetate	1298	-	-	0.1	MS, LRI
trans-Sabinyl acetate	1316	-	-	0.3	MS, LRI
Myrtenyl acetate	1329	1663	-	1.7	MS, LRI
α-Cubebene	1355	1460	0.1	-	MS, LRI
α-Copaene	1382	1496	0.3	0.2	MS, LRI
β-Bourbonene	1390	1518	0.4	0.2	MS, LRI
α-Gurjunene	1420	-	0.1	-	MS, LRI
(*E*)-*β* Caryophyllene	1428	1576	**9.2**	**12.3**	GC, MS, LRI
β-Copaene	1434	-	0.1	-	MS, LRI
(*Z*)-*β*-Farnesene	1443	1659	**6.9**	-	MS, LRI
α-Humulene	1462	1642	1.7	**5.1**	GC, MS, LRI
allo-Aromadendrene	1467	-	-	0.1	MS, LRI
Germacrene D	1490	1687	**4.4**	1.7	MS, LRI
β-Selinene	1493	-	-	0.1	MS, LRI
epi-Cubebol	1494	1927	0.3	-	MS, LRI
Valencene	1496	1693	-	0.3	MS, LRI
Bicyclogermacrene	1510	1710	1.3	-	MS, LRI
Cubebol	1522	1956	0.4	-	MS, LRI
γ-Cadinene	1522	1748	0.5	0.1	MS, LRI
δ-Cadinene	1530	1735	0.5	0.4	MS, LRI
(*E*)-Nerolidol	1562	2039	**16.5**	-	GC, MS, LRI
Caryophyllene oxide	1590	1956	-	1.6	MS, LRI
Guaiol	1602		0.3	-	MS, LRI
Humulene epoxide II	1620	2024	-	0.4	MS, LRI
α-Cadinol	1664	2218	0.1	-	MS, LRI
Caryophylla-4(12),8(13) dien-5-ol	1643	2276	-	0.1	MS, LRI
Epi-α-muurolol	1646	2327	-	0.4	MS, LRI
14-hydroxy-9-epi (*E*)-Caryophyllene	1667	2357	-	0.1	MS, LRI
Total			**99.2%**	**99.7%**	

The components and the percentages are listed in order of their elution on the apolar column (HP_5)

a = Linear retention indices on HP_5 column (a 5%-Phenyl-methylpolysiloxane phase); temperature program 60-200°C at 3°C/mn, then 200°C for 20 min.

b = Linear retention indices on Supelcowax 10 (polyethylene glycol phase) temperature program 50-200°C at 5°C/min, then 200°C for 10 min.

Methods of identification: GC, identification based on co-injection with authentic sample, MS, identification based on comparison of mass spectrum with literature data, LRI, identification based on comparison of retention index with those of published data.

collected in Korhogo [19a] while another sample harvested in Bouaké was dominated by (*Z*)-nerolidol (45.2%), linalool (20.2%) and (*Z*)-β-farnesene (10.5%) [19b]. The intraspecific chemical variability of the leaf oil of *L. multiflora* from different areas of Ivory Coast was investigated by GC and Carbon-13 NMR spectroscopy [20]. Four chemical compositions could be distinguished, which were characterized by citral or 1,8-cineole associated to citral /sabinene-α-terpineol/ linalool in variable relative proportions. Finally, the most recent study reports on the bactericidal activity of an essential oil from *L. multiflora* dominated by 1,8-cineole (48.9%) associated with geranial (15.1%) and on its potential application as anti-diarrheic ingredient [21]. The large chemical variability previously described justified new investigations on the species growing in Ivory Coast.

The Zingiberaceae is a tropical monocotyledon family comprising some 1300 species, many of which produce essential oils often in their leaves and rhizomes [22a]. Traditionally, *A. latifolium* K. Schum is used in Cameroon for the treatment of malaria symptoms [22b-22d]. A bioguided fractionation of its fruits extracts resulted to isolation of labdanes which showed a modest *in vitro* activity against a chloroquine sensitive *Plasmodium falciparum* strain [23] while the essential oil obtained from the fruit husk exhibited a significant antifungal activity against *Aspergillus flavus* [24]. Finally, the essential oils obtained from leaves of *A. latifolium* collected in Cameroon were characterized by hydrocarbon terpenoids of which α and β-pinenes (51.7%-86.6%) were the major components [22a,25a]. Up to now, *A. latifolium* essential oils have never been investigated in Ivory Coast although the rhizomes of the plants are often used in food and traditional medicine.

Some studies have been reported on the insecticidal activity of the essential oil of *L. multiflora*. This oil has demonstrated promising larvicidal activities against *Anopheles gambiae* and *Aedes aegyti* larvae in Burkina Faso [25b]. On the other hand, among the aromatic plants investigated in Togo for *Callosobruchus maculatus* pest control [26], the essential oil obtained from *L. multiflora* showed only a moderate insecticidal activity. About ten years later, the same species was tested again, along with three other aromatic plants, regarding optimization of their essential oil use to protect stored cowpeas [27a]. A significant pediculocidal and scabicidal activity was demonstrated for an essential oil obtained from leaves of *L. multiflora* collected in Nigeria [27b]; the essential oil was dominated by linalool (26.7%), geraniol (20.4%) and limonene (15.4%). Nevertheless, a few reports on use of essential oils against phytophagous insects are available [28].

In the present paper, the results of our chemical investigations on the essential oils from two aromatic plants, *Lippia multiflora* Mold. (Verbenaceae) and *Aframomum latifolium* K. Schum (Zingiberaceae) collected in Ivory Coast are given; the insecticidal activity of both volatile extracts was tested *in vitro* against the polyphagous *B. tabaci*.

The essential oil content was higher for *L. multiflora* than for *A. latifolium* (Table 1). These results are comparable to those previously obtained for the species *L. multiflora* from Ivory Coast (between 0.5 and 1.18%) [22a,29a] while leaves of *A. latifolium* from Cameroon furnished essential oil with only 0.05% yield [25a]. The relative density and refractive index measured at 20°C are similar for both samples; the values measured for the essential oil of *L. multiflora* are close to those previously reported [18,29a].

The results of our chemical analyses performed by GC-FID and GC-MS are presented in Table 2, where the compounds are listed according their order of elution on a DB5 column. Both oils possessed quite different chemical compositions, even if they were exclusively terpenic.
The essential oil of *L. multiflora* was dominated by oxygenated terpenoids, with two major acyclic derivatives, linalool (46.6%) and its sesquiterpene analogue (*E*)-nerolidol (16.5%). The essential oil of *A. latifolium* contained a majority of hydrocarbon terpenoids, mainly β-pinene (51.6%) and α-pinene (6.7%). In both cases, (*E*)-β-caryophyllene was the main hydrocarbonated sesquiterpene (9.2% and 12.3% respectively) accompanied by α-humulene (1.7-5.1%), germacrene D (1.7-4.4%); (*Z*)-β-farnesene (6.9%) was only found in the *L. multiflora* essential oil.

The chemical profile of the leaf essential oil of *A. latifolium* is similar to those previously described for samples originating from Cameroon [22a,25a] with nevertheless some qualitative and quantitative differences. The chemical composition of *L. multiflora* essential oil is representative of the chemotypes dominated by acyclic terpenoids [29b]; it is nevertheless quite different from those previously reported: the association linalool/(*E*)-nerolidol in high amounts (46.6%/16.5%) is described for the first time in the *Lippia* genus.

The toxicity of the essential oils against *B. tabaci* at the six doses tested (0.4 to 3.4 µL/L air) when the *B.tabaci* adults were exposed to their vapors for 24h is shown in Table 3. With the *L. multiflora* oil, the mortality was maximal from the lower dose (0.4 µL/L air). With the *A. latifolium* oil, the mortality increased significantly from 90.8% with the

Table 3: Toxicity (expressed in percent mortality) of essential oils vapors against adults of *B. tabaci* after 24 hours exposure at room temperature

Essential oils	**Dose (µL/L air)**						
	0	**0.4**	**1**	**1.6**	**2.2**	**2.8**	**3.4**
L. multiflora	5.1±2.4	97.4±4.5	98.0±1.7	98.4±1.4	98.9±1.7	99.3±1.2	100
A. latifolium	2.4±2.4	90.8±2.0	92.7±6.4	94.2±2.2	98.9±1.7	99.2±1.4	99.9±1.9

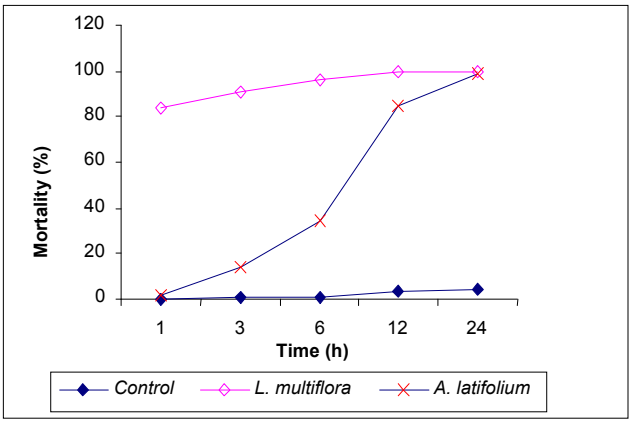

Figure 1: Effect of essential oils of *L. multiflora* and *A. latifolium* on adults of *Bemisia tabaci* as a function of exposure time at a dose of 0.4 µL /L air.

Table 4: Lethal time in hours (LT_{10}, LT_{50}, LT_{90}) of essential oils of *L. multiflora* and *A. latifolium* against adults of *Bemisia tabaci* (exposure to 0.4 µL /L air)

Lethal time (h)	*Essential oils*	
	L. multiflora	*A. latifolium*
LT_{10}	< 1	3.48±0.94
LT_{50}	< 1	**7.18±0.93**
LT_{90}	1.87±0.94	15.07±1.40

lower dose to the maximum obtained with 2.2 µL/L air. Both essential oils samples significantly reduced adult survival relative to control. The high sensitivity of adults of *B. tabaci* led us to evaluate the effect of the lowest dose (1µL of essential oil deposited on a filter paper) for different exposure times. More than 80% mortality was observed in the case of exposure of insects to *L. multiflora* essential oil vapors during 1 hour while the same toxicity was obtained after 12 hours in the case of *A. latifolium* (Figure1). The lethal times (LT_{10}, LT_{50} and LT_{90}) inducing 10, 50 and 90% mortality were presented in Table 4.

These results showed the high insecticidal action of *L. multiflora* essential oil against *B. tabaci* adults compared with *A. latifolium*. The effectiveness *of L. multiflora* oil against *B. tabaci* adults was much higher than that previously observed against *Callosobruchus maculatus* in Togo [26]. These preliminary and promising results will be confirmed and complemented in the future by evaluation of the contact toxicity of these essential oils and of their repellent potentialities against various insect species.

The insecticidal activity of an essential oil depends on its chemical composition and of the sensitivity of the target pest to its active compounds. The essential oil of *L. multiflora* which was the most efficient contained a high proportion of oxygenated components (linalool, (*E*)-nerolidol) while the volatile extract of *A. latifolium* was dominated by hydrocarboned monoterpenes (mainly pinenes). Furthermore, it was demonstrated that oils with a high proportion of hydrocarbon components lost their activity more rapidly than those containing mainly oxygenated compounds [30].

In conclusion, the essential oils from *L. multiflora* and *A. latifolium* seemed to be highly active on the whitefly *B. tabaci* and could play a role as fumigants in greenhouses. Or indirectly, in some agroecosystem, the pest could be deterred or repelled from the crop by push stimuli delivered by intercropping with non-host plant with deterrent or repellent attributes [31]. Further experiments should be necessary for optimization of their use as agroecological alternative for crop protection.

Experimental

Sources and extraction of essential oils: The plant material consists of leaves of *Lippia multiflora* and *Aframomum latifolium* harvested in April 2008 in the town of Yamoussoukro (Ivory Coast). The plants were identified at the Centre National de la Flora (CNF) at the University of Cocody-Abidjan by Laurent Aké Assi. The plant material used for isolation of the essential oils was air-dried at room temperature (28 ± 2°C). Fractions of each dried plant material (500 g) were submitted to steam distillation using a Clevenger type apparatus for 3 hours. Anhydrous magnesium sulphate was used to remove residual water after decantation. Essential oils were stored in tightly closed dark vial at 4°C until the analyses and tests. The yields were calculated according to the weight of the plant material before distillation (w/w of the dry vegetable material, expressed in percentage).

Physical characteristic: They are mainly the refractive index (n) and the density (d). The measurement of (n) was performed using an electronic refractometer brand *Leicia AR200* after calibration with distilled water. The density (d) of the oils was determined relatively to distilled water by using a pycnometer.

Analysis: The GC analyses were performed on a Varian gas chromatograph, model CP-3380, with flame ionization detectors equipped with two silica capillary columns: HP5 J&W Agilent (5%-Phenyl-methyl polysiloxane) capillary column (30 m x 0.25 mm i.d. x 0.25 µm film) and Supelcowax 10 (polyethylene glycol) fused capillary column (30 m x 0.25 mm i.d. x 0.25 µm film); N_2 was the carrier gas at 0.8 mL/min; injection type 0.1 µL of pure sample, split ratio 1:100; injector temperature 220°C, detector temperature 250°C; temperature program 60-200°C at 3°C/min, then kept at 200°C during 20 minutes (HP5) and 50-200°C at 5°C/min, then kept at 10 minutes (Supelcowax). The linear retention indices of the components were determined relatively the retention times of a series of *n*-alkanes. The GC-MS analyses were performed using a Hewlett–Packard GC 5890 series II equipped with a HP5 (5%-Phenyl-methyl polysiloxane) fused silica column (30 m x 0.25 mm; film thickness 0.25 µm) and a DB-Wax fused silica column (30 m x 0.25 mm; film thickness 0.25 µm) interfaced with a quadrupole detector (Model 5972); temperature program (60-200°C at 3°C/min); injector temperature, 220°C; MS transfer line temperature, 180°C; carrier gas, helium at a flow rate of

0.6 mL/min; injection type, split, 1:10 (1 µL 10:100 CH_2Cl_2 solution); ionization voltage, 70 eV; electronmultiplier 1460 eV; scan range 35-300 amu; scan rate, 2.96 scan/s.

The identification of the constituents was based on comparison of their relative retention times with either those of authentic samples or with published data in the literature [32] and by matching their mass spectra with those obtained from authentic samples and/or the NBS 75K and Wiley 7th NIST 98 EPA/NIH libraries spectra and literature data [32]. The results are reported in Table 2. The percentage compositions were obtained from electronic integration measurements from the GC-FID chromatograms on the apolar column without taking into account the relative response factors.

Insect rearing: Insect culture of *B. tabaci* were maintained in the laboratory on cotton plants (*Gossypium hirsutum*) grown in cages (plastic pots 20 x 16 x 18.5 cm containing local soil). Rearing conditions were 28±2°C and 60±5% relative humidity at photoperiod of 12: 12 hours L/D.

Bioassays: Toxicity of the essential oils was tested against the adults of *B. tabaci* by using fumigation method in a glass desiccator (volume 2.5 liter) [33]. The desiccators were separated in two compartments (1 and 2) by a perforated grid. For each essential oil, a series of sample concentrations was prepared by using a micropipette. A filter paper (Whatman n°1, 4.2 cm diameter) was treated with 1 µL, 2.5 µL, 4 µL, 5.5 µL, 7 µL and 8.5 µL of oil and placed at the bottom of the desiccator (compartment 2) from which the vapors of oil diffused in the whole chamber Thus the 6 doses tested varied from 0.4 to 3.4µL/L air. A glass Petri dish (9 cm diameter) containing a cotton leaf disc on Agar-agar was then deposited in the compartment 1 on the grid. The equilibrium time was 5 minutes at 28±2°C and 60 ± 5% relative humidity. Forty newly emerged adults (<24h) aspirated into a small tube were deposited onto each leaf disc in the Petri dish and the desiccator was immediately closed and sealed with wax. The desiccators were held at 28 ± 2°C for 24h, after which, the mortality of insects was assessed using a binocular microscope. Adults showing no sign of movement when lightly shaked by a brush were scored as dead. For control, the same protocol was applied without oil. Each experiment was repeated three times. Owing to the high sensitivity of adults *B. tabaci* against both oils, the insects were exposed at the lowest dose (0.4 µL/L air), during various times: 1h, 3h, 6h and 12h. The lethal times (LT_{10}, LT_{50} and LT_{90}) were calculated using the software biostatistics Win DL version 2.0 CIRAD-CA U.R.B.I. / M.A.B.S. October 1999.

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