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Abstract

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a major pest of many agricultural and ornamental crops in tropical and subtropical regions causing damages that result in important economic losses. Insecticides are commonly used in greenhouses or fields to control *B. tabaci* populations leading to rapid evolution of resistance that render treatments inefficient. Therefore, and for environmental and human health concerns, other approaches must be developed for this pest management. In the present study, we compare, using the leaf dip method, the toxicity of three essential oils (*Cymbopogon citratus*, *Ocimum americanum*, and *Hyptis spicigera*) and three seed oils (*Lannea microcarpa*, *Lannea acida*, and *Carapa procera*) with three chemical insecticides (acetamiprid, deltamethrin, and chlorpyrifos-ethyl) on adults. Two *B. tabaci* biotypes (MED-Q1 and MED-Q3) belonging to the Mediterranean species and collected in Burkina Faso were used. Essential oils were analyzed by gas chromatography–mass spectrometry and gas chromatography–flame ionization detector. We showed that these two biotypes have different levels of resistance to the three insecticides, MED-Q3 being more sensitive than MED-Q1. Moreover, they differ in the frequency of resistance alleles to insecticides, especially for organophosphates, as these alleles are almost fixed in MED-Q1. On the other hand, the two biotypes prove to be more susceptible to the plant extracts than to insecticides except for chlorpyrifos-ethyl, with essential oils that showed the highest insecticidal activities. Monoterpenes content were the most abundant and showed the highest insecticidal activities. Our results indicated that essential oils, but also seed oils, have the potential to constitute an alternative strategy of pest management.

Key words: *Bemisia tabaci*, essential oil, seed oil, insecticide resistance, leaf dip method

The sweet potato whitefly, *Bemisia tabaci* (Gennadius), is one of the most serious pests of many agricultural and ornamental crops in tropical and subtropical regions (Perring 2001, De Barro et al. 2011). It is a cryptic species complex which differs in some biological characteristics such as host range, fecundity, insecticide resistance, ability to transmit plant viruses, induction of plant disorders (Perring 2001, Simmons et al. 2008, De Barro et al. 2011), and the symbiotic bacterial community (Gueguen et al. 2010). Recently, Lee et al. (2013) described 31 putative species, among which the Middle East-Asia Minor 1 (MEAM1), known commonly as biotypes B and B2, and the Mediterranean (MED), known as biotypes Q, J, L, and the sub-Saharan Africa Silverleaf biotype species (ASL), are recognized as predominant in many areas (Dinsdale et al. 2010).

This insect is extremely polyphagous, with 600 species of host plants described at the time (Oliveira et al. 2001, Evans 2007, Simmons et al. 2008, Li et al. 2011). *Bemisia tabaci* induces severe damage directly by feeding, but also, indirectly through the development of fungal infection on leaves owing to the honeydew excretion (Oliveira et al. 2001) or the transmission of several hundred plant viruses (Jones 2003, Liu et al. 2013). The resulting losses have a severe impact on the economic activity. As a consequence, pesticides, such as organophosphates, neonicotinoids, and pyrethroids, are commonly used to treat crops in greenhouses and open fields despite

their harmful impact on potential natural enemies (Gnankiné et al. 2005), on the environment, and on the human health. This practice also leads to high levels of resistance that can appear rapidly in whiteflies because of their high fecundity and short generation time (Denholm et al. 1998, Perring 2001). For example, populations of *B. tabaci* resistant to neonicotinoids and carbosulfans have been observed in several Western African countries where repetitive and intensive insecticide treatments are practiced, particularly on cotton and vegetables (Houndété et al. 2010a,b; Gnankiné et al. 2013a,b). Mutations in the *para*-type voltage-gated sodium channel gene (L925I and T929V) and in the acetylcholinesterase enzyme *ace1* gene (F331W) have been identified to confer resistance to pyrethroids and organophosphates, respectively (Morin et al. 2002; Alon et al. 2006, 2008; Roditakis et al. 2006). As a consequence, these insecticides, which act on the insect nervous system and induce insect paralysis and death, become inefficient. Moreover, it has been shown that they have a negative impact on the populations of natural enemies of *B. tabaci* (Gnankiné et al. 2005, Simmons et al. 2011). Therefore, there is a real need to develop efficient, safer, and environmentally friendlier alternative methods that have the potential to replace chemical pesticide in terms of *B. tabaci* control.

Several research programs are focused in the search of natural pesticides such as plant extracts, and precisely, essential oils extracted from aromatic plants. These oils have many applications in cosmetics, perfumeries, detergents, pharmacy, fine chemicals, and food production (Bakkali and Idaomar 2008). Some essential oils have also been recognized for their antimicrobial and insecticidal properties (Regnault-Roger et al. 2012). The effect of essential oils and aqueous extracts on *B. tabaci* has been investigated in different studies (Calmasur et al. 2006, Cloyd et al. 2009, Yang et al. 2010, Yarahmadi et al. 2013, Tia et al. 2013, Chae et al. 2014, Emilie et al. 2015, Deletre et al. 2016). Results showed repellent, irritant, and toxic effects of essential oils on whiteflies. These effects are variable according to the plant extract and concentration, but these data indicate that they can be good candidates for alternative strategies for the control of *B. tabaci* populations. On the other hand, at the time, there were no attempts to study the toxicity of other oils on crop pests like seed oils.

In the present study, the toxicity of three essential oils (*Cymbopogon citratus* (DC), Stapf, *Ocimum americanum* L., and *Hyptis spicigera* Lam.) and three seed oils (*Lannea microcarpa* Engl. & K. Krause, *Lannea acida* A. Rich, and *Carapa procera* DC) and three chemical insecticides (a neonicotinoid: acetamiprid, a pyrethroid: deltamethrin, and an organophosphate: chlorpyrifos-ethyl) to adult *B. tabaci* was compared. This study was performed on field populations of *B. tabaci* from Burkina Faso in West Africa, for which there is no data available on their susceptibility to plant extracts. In this country, three biotypes have been described, all belonging to the MED species-MED-Q1, MED-Q3, and MED-ASL (Gnankiné et al. 2013c). MED-Q1 and MED-ASL coexist sometimes in the same area (Gnankiné et al. 2013d) but differed highly in frequencies of resistance alleles to insecticides, which were much higher in Q1 than in ASL (Mouton et al.2015). Up to now, MED-Q3 was found only on *Lantana camara* in Ouagadougou located in the middle part of Burkina Faso (Gnankiné et al. 2013d).

Individuals were collected on two host plants-a cultivated vegetable, tomato, and an ornamental plant, *L. camara*, the first being subject to intensive insecticide treatments and not the other. This study also investigates the presence of alleles associated with insecticide resistance. The data obtained will provide important information on the possibility to use plant extracts to control *B. tabaci* populations in Burkina Faso.

Materials and Methods

Whitefly Populations

Between 2014 and 2015, 11,340 adults of *B. tabaci* were sampled in two locations, on two host plants in Burkina Faso-on *Lantana camara* in the University of Ouagadougou and on tomato plants (*Solanum lycopersicum*) in Tanghin (Fig. 1). Chemical insecticides are currently used on tomato but not on *L. camara*. In each area, adult *B. tabaci* were collected randomly from 80 plants using a mouth aspirator, confined in a wooden rearing cage (50 by 35 by 35 cm) containing tomato and *L. camara* seedlings, and returned to the laboratory within 2–5 h. Experiments were performed on whiteflies of both sexes the same or the following day. For molecular analysis, individuals were conserved in alcohol (80%) at −20 °C until use.

Determination of *B. tabaci* Biotypes

Total DNA was extracted from individual adults of *B. tabaci* in 26 µl of an extraction buffer containing 50 mM of KCl, 10 mM of Tris-Base pH 8, 0.45% Nonidet, 0.45% Tween 20, and 50 mg/ml of proteinase K, as described in Delatte et al. (2005). Biotypes were identified using the Polymerase Chain Reaction-Random Fragment Length Polymorphism (PCR-RFLP) diagnostic assay based on the mitochondrial marker cytochrome oxidase 1 gene sequence (*mtCO1*) described in Henri et al. (2013). This method allows discriminating all the known biotypes detected in West Africa (Gnankiné et al. 2013b).

Identification of Susceptible and Resistant Alleles of the Sodium Channel and *ace1* Genes

Resistant (I925) and susceptible (L925) alleles in the *para*-type voltage-gated sodium channel as well as *ace1*-susceptible (F331) and *ace1*-resistant (W331) alleles were detected using PCR-RFLP assays, as described in Tsagkarakou et al. (2009).

Briefly, genomic DNA amplification with the two primers Bt-ace-F (5′-TAGGGATCTGCGACTTCCC-3′) and Bt-ace-R (5′-GTTC AGCCAGTCCGTGTACT-3′) produced a 201-bp fragment, which is cut by BsrI into two fragments for susceptible homozygotes (*ace-1 SS*) and three fragments for resistant homozygotes (*ace-1 RR*). Heterozygous individuals (*ace-1 RS*) display a combined pattern.

As for kdr detection, genomic DNA amplification with the two primers Bt-kdr-F1 (5′-GCCAAATCCTGGCCAACT-3′) and Bt-kdr-RIntr1 (5′-GAGACAAAAGTCCTGTAGC-3′) produced a 184-bp fragment, which is undigested by DdeI for resistant homozygotes (*kdr-1 RR*), and cut into two fragments for susceptible homozygotes (*kdr-1 SS*) and three fragments for heterozygous (*kdr-1 RR*).

The frequencies of *kdr* and *ace-1*^{R} mutations were calculated according to the formula$p = n♂(R)+2n♀(RR)+n♀(RS)/n♂+2n♀$

where RR was the number of homozygotes, RS the number of heterozygotes, and *n* the size of specimens analyzed.

Insecticides

The following chemicals-deltamethrin (Deltacal 12.5 g/liter), acetamiprid (Titan 25 g/liter), and chlorpyrifos-ethyl (Pyrical 480 g/liter) provided by Saphyto (Ouagadougou, Burkina Faso) were used for the bioassays in the laboratory.

Seeds and Seed Oil Extraction

Mature fruits of *L. microcarpa*, *Lannea acida*, and *Carapa procera* (1.5 kg each) were collected on the ground in Djanga (10° 37′ N, 004° 13′ W) and Bérégadougou (10° 49′ N, 004° 47′ W), Burkina Faso. The plant species were identified and voucher specimens were

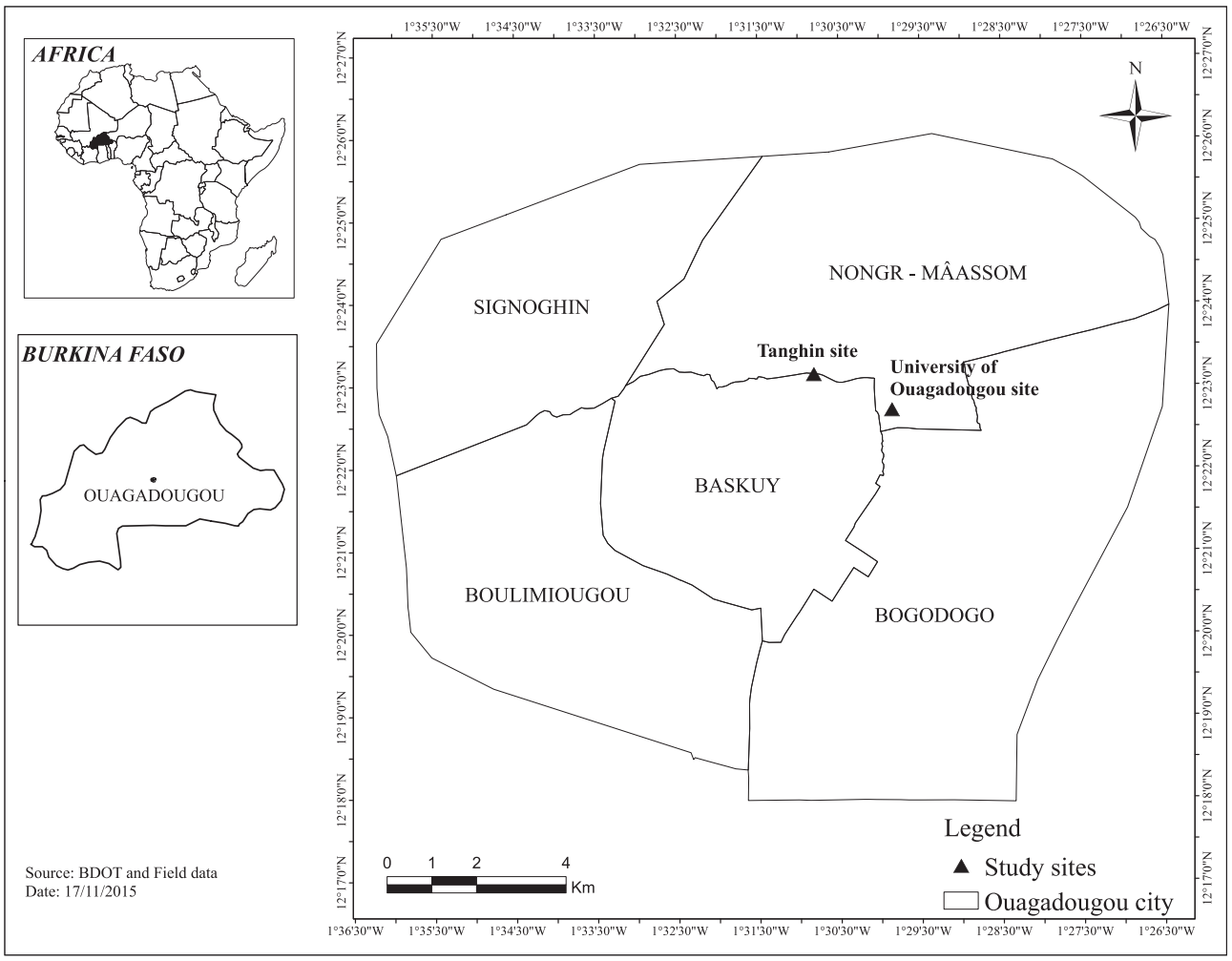

Fig. 1. Map of the sampling sites.

deposited at the herbarium of laboratory of plants biology and ecology of University Ouaga I Pr Joseph KI-ZERBO (Burkina Faso). Seeds were hand sorted to eliminate damaged ones before taking to the laboratory. Prior to any analysis, the samples were washed with glass-distilled water, drained, and air-dried at the laboratory condition (22–23 °C) during 1 wk. The outer dried tissue surrounding the pith of the fruit was removed and seeds were decorticated and fine ground with a Moulinex grinder robot (GT550, Zurich, Switzerland).

Seed oils were extracted using the Soxhlet extraction (American Oil Chemists's society [AOCS] 1990) apparatus with petroleum ether (40–60 °C) as solvent for 6 h. Oils were separated from the organic solvent using a rotary vacuum evaporator. Immediately after evaporation of extract solvent, oils were saturated with nitrogen air and stored at −18 °C.

Plant Materials and Essential Oil Extraction

The aerial parts of *Cymbopogon citratus* (DC), Stapf, *Ocimum americanum* L., *and Hyptis spicigera* were collected from the botanical garden at the Institut de Recherche en Sciences Appliquées et Technologies (IRSAT), Ouagadougou, Burkina Faso. Samples were air-dried at the laboratory condition (22–23 °C) during 1 wk. For each sample, about 200 g of dried material were submitted to hydrodistillation using a Clevenger-type apparatus for 3 h. Essential oils were dessicated using anhydrous sodium sulphate and stored in airtight containers in a refrigerator at 4 °C.

Gas Chromatographic/Flame Ionization Detector (GC/FID) Analysis

Essential oils obtained from aerial parts of *Cymbopogon citratus*, *Ocimum americanum*, and *Hyptis spicigera* were analyzed using an Agilent 6890N gas chromatograph, with a DB-5 narrow bore column (length 10 m × 0.1 mm ID, 0.17 µm film thickness; Agilent, Palo Alto, CA) equipped with a flame ionization detector. Helium (average velocity of 42 cm/s) was used as the carrier gas. Oven temperature was programmed from 60°C to 165 °C at 8 °C/min and from 165°C to 280 °C at 20 °C/min, with 1 min post run at 280 °C. Diluted samples (1/100 in acetone) of 1.0 µl were injected manually and splitless. Peak area percentage was calculated on the basis of the FID signal using the GC HP-Chemstation software (Agilent Technologies).

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

Gas Chromatography/Mass Spectrometry analysis was carried out on a HP 6890 GC coupled to HP 5972 MSD (Hewlett Packard, Palo Alto, CA), and was equipped with a ZB-5MS Zebron capillary column (length 30 m × 0.25 mm ID, 0.25 µm film thickness; Agilent).

Helium was the carrier gas. The oven temperature was held at 45 °C for 2 min and increased from 45°C to 165 °C at 4 °C/min, and from 165°C to 280 °C at 15 °C/min. Samples (1 µl) were injected at 250 °C and the split ratio was 50:1.

Identification of Components

Identification of the essential oil components were carried out by comparison of their retention indices with those of homologous series of n-alkanes (C8–C32) determined under the same operating conditions, and by comparison of their mass spectra with those of literature data (Adams 2007, Stein et al. 2002). Component relative percentages were calculated without using correction factors.

Bioassays

Serial dilution of chemical and essential and seed oil extracts were made, respectively, in distilled water and 70% ethanol. Test concentrations varied from 5 to 500 mg/liter, 4 to 14 mg/liter, and 0.8 to 6 mg/liter for chemical, seed, and essential oils, respectively. Details of assay protocol and data analysis were already published by Gnankiné et al. (2013a).

Adult whitefly tests were performed using a leaf dip bioassay method (Rowland et al. 1991, Cahill et al. 1995, Horowitz et al. 2005). For each insecticide, discs of *L. camara* (35 mm in diameter) and tomato (whole leaf) leaves were immersed for 10 s in nine aqueous solutions of various concentrations of insecticide and plant extracts (from the lowest to the highest concentrations), or in distilled water and 70% ethanol (controls). The leaf discs were air dried for 30 min before being placed individually on an agar gel (1%) in a vial (45 mm in diameter). Thirty individuals of both sexes of *B. tabaci* were removed from *L. camara* and tomato leaves with a mouth aspirator. Then, adults of *B. tabaci* were chilled and transferred into small plastic vials containing the treated leaf discs. Afterwards, each petri dish was closed with a transparent ventilated lid. When adults were recovered from chilling, petri dishes were kept upside down and maintained in a climatic room at 25 ± 2°C, 60 ± 5% relative humidity (RH), and a photoperiod of 12:12 (L:D) h. Alive insect was recognized when any sign of movement was noted. Mortality was assessed 24 h later. Three replicates were carried out for each concentration of each product and also for controls.

Statistical Analyses

All bioassay replicates were used for analysis. The software XLSTAT 2015.1.01 allowed us to generate lethal concentrations (LC_{50}), slope values, and the respective 95% confidence limits. A population is considered to be significantly more (or less) resistant than another population when no overlap of the 95% confidence limits of the LC_{50} is recorded. Mortality in the control was always <10%. Overall, all bioassays data were corrected for control mortality using Abbott's formula (Abbott 1925).

Results

Chemical Composition of the Essential Oils

Monoterpenes were the most abundant volatiles detected in the essential oils (Table 1). Oxygenated monoterpenes were the most dominant in the essential oils of *Cymbopogon citratus* and *Ocimum americanum*, whereas monoterpene hydrocarbons were the most abundant in the essential oil of *Hyptis spicigera*. *Cymbopogon citratus* essential oil was characterized by the presence of five components, representing 98.1%. Geranial (48.1%), neral (35.8%), and myrcene (11.0%) were the major constituents. Geraniol (2.5%) and linalool (0.7%) were the minor constituents. In the essential oils of *H. spicigera*, 22 compounds, accounting for 97.2% of total compounds, were identified. Major compounds were β-caryophyllene (22.15%), α-pinene (20.11%), sabinene (10.26%), β-pinene (9.22%), α-phellandrene (7.03%) and the minor were terpinolene (4.43%), α-thujene (4.37%), *p*-cymene (3.05%), Germacrene-D (2.75%), β-phellandrene (2.69%), Limonene (2.33%), α-humulene (1.89%), eucalyptol (1.81%), caroyphyllene oxide (1.4), and elemol (1.25%). In total, 41 constituents were identified in the essential oil of *O. americanum*, representing 99.75% of the total oil. The most abundant compounds were eucalyptol (41.4%), camphor (10.8%), and α-pinene (9.6%). The minor compounds were limonene (4.77%), *trans*-α-bergamotene (3.68%), camphene (3.66%), bornyl acetate (3.05%), α-cayophyllene (2.42%), α-terpineol (1.77%), myrcene (1.43%), (ZE)-α-farnesene (1.31%), *p*-cymene (1.31%), and sabinene (1.25%).

Identification of *B. tabaci* Biotypes

All the 25 individuals collected on tomato and *L. camara* belonged to the Mediterranean (MED) species of the *B. tabaci* species complex, as previously described in Burkina Faso (Gnankiné et al. 2013c). Two of the three biotypes found in the previous study have been detected-MED-Q1 on tomato and MED-Q3 on *L. camara*.

Detection of Sodium Channel and *ace1* Resistant Mutations and Allele Frequencies

The PCR-RFLP tool developed by Tsagkarakou et al. (2009) was used to check for the presence of resistant mutations in the *ace1* (F331W) and in the *para*-type voltage-gated sodium channel (L925I and T929V) genes in the samples collected. For both genes, the frequencies of resistant alleles were variable, depending on the biotype. For the *para*-type voltage-gated sodium channel gene, only the mutation L925I was found (allele called r1 by Alon et al. 2008), with a frequency of 0.96 and 0.8 in MED-Q1 and MED-Q3 biotypes, respectively. On the other hand, the F331W mutation in the *ace1* gene was almost fixed in MED-Q1 population, while it was absent in MED-Q3 individuals (Table 2).

Susceptibility of *B. tabaci* Adults to Insecticide, Essential and Seeds Oils

Globally, Q3 individuals are more sensitive to chemical pesticides than Q1 individuals. Overall, the two biotypes were more susceptible to the plant extracts than to the chemical insecticides except for chlorpyrifos-ethyl (organophosphate). Among the plant extracts, essential oils showed the highest toxicity (Table 3).

The report of LC_{50} values determined confirm the higher toxicity of essential oils compared with the insecticides. For example, essential oil extracted from *Hyptis spicigera* was 18.66-fold, 63.58-fold, and 180.75-fold toxic than chlorpyrifos-ethyl, deltamethrin, and acetamiprid, respectively, in Q1 population.

For the biotype MED-Q1, the neonicotinoid acetamiprid is, by far, the least toxic (LC_{50}:121.1 mg/liter) followed by deltamethrin exhibiting an LC_{50} value of 42.6 mg/liter. The highest susceptibility was observed for Chlorpyrifos-ethyl both for MED-Q1 and MED-Q3 (LC_{50}: 12.5 mg/liter for MED-Q1 and 3.7 mg/liter for MED-Q3). Among seed oils, there is no significant difference between the three plant extracts in neither of the two biotypes. LC_{50} value was between 4.7 to 6.9 mg/liter for MED-Q1 and around 2.5 for MED-Q3. The LC_{50} values for essential oils varied from 0.67 to 1.5 mg/liter

Table 1. Chemical composition of *Cymbopogon citratus*, *Hyptis spicigera*, and *Ocimum americanum* essential oils

Compounds	Retention time (min)	Proportion (%)		
		Cymbopogon citratus	*Hyptis spicigera*	*Ocimum americanum*
Hydrocarbon monoterpenes				
Tricyclene	9.7			0.12
γ-Terpinene	15			0.21
α-Thujene	9.88		4.37	0.18
α-Pinene	10.16		20.11	9.64
Camphene	10.84		0.09	3.66
Sabinene	11.78		10.26	1.25
β-pinene	11.94		9.22	5.69
Myrcene	12.48	11	0.81	1.43
δ-2-Carene	12.78			0.15
α-Phellandrene	13.1		7.03	
α-Terpinene	13.46		0.61	
p-Cymene	13.78		3.05	1.31
Limonene	13.94		2.33	4.77
β-Phellandrene	13.95		2.69	
(E)-β-Ocimene	14.47		0.13	
Terpinolene	15.92		4.43	0.18
Total		11	65.13	28.59
Hydrocarbon sesquiterpenes				
α-Copaene	24.54			0.16
β-Elemene	24.88			0.17
β-Caryophyllene	25.12		22.15	
α-Caryophyllene	25.68			2.42
Trans-α-Bergamotene	25.98			3.68
(Z)-β-Farnesene	26.46			0.11
α-Humulene	26.58		1.89	0.16
Germacrene-D	27.2		2.75	0.08
Trans-β-Bergamotène	27.26			0.25
(ZE)-a-Farnesene	27.38			1.31
α-Muurolene	27.6			0.14
δ-Cardinene	28.08			0.31
Total			26.79	8.79
Oxygenated monoterpenes				
Eucalyptol	14.1		1.81	41.42
Cis-sabinene hydrate	15.48			0.30
Linalool	16.5	0.7		0.74
1-octen-3-yl acetate	16.76			0.64
Camphor	18.04			10.84
α-Terpineol	18.76			0.38
Terpinen-4-ol	19.06		0.75	0.62
α-Terpineol	19.52			1.77
α-Fenchyle acetate	20.18			0.11
Neral	21.13	35.8		
Piperitone	21.28		0,08	0.58
Bornyl acetate	22.1		0.05	3.05
Geraniol	22.12	2.5		
Isobornyl acetate	22.16			0.15
Geranial	22.95	48.1		
Myrtenyl acetate	23.16			0.67
Exo-2-hydroxycineole acetate	23.58			0.11
Total		87.10	2.69	61.38
Oxygenated sesquiterpenes				
Elemol	28.8		1.25	0.34
(E)-Nerolidol	29.08			0.13
Caroyphyllene oxide	29.62		1.4	0.33
α-Eudesmol	31.26			0.19
Total			2.65	0.99
Monoterpene hydrocarbon		11.00	65.13	28.89
Sesquiterpene hydrocarbon		0.00	26.79	8.80
Oxygenated monoterpene		87.10	2.69	61. 38
Oxygenated sesquiterpene		0	2.65	0.99
Total		98.10	97.26	99.75

Table 2. Frequencies of sodium channel and ace-1 resistance mutations in *B. tabaci* MED-Q1 and MED-Q3 biotypes from populations collected in Burkina Faso

Biotype	Ace-1 (Locus F331W)								Sodium channel (Locus L925I)							
	Males' genotypes			Females' genotypes				Allele frequencies	Males' genotypes			Females' genotypes				Allele frequencies
	n	r1	s	*n*	r1r1	r1s	ss		*n*	R	S	*n*	RR	RS	SS	
MED-Q1	3	3	0	12	11	1	0	r1 = 0.98	3	3	0	12	10	2	0	R = 0.96
MED-Q3	0	0	0	10	0	0	10	r1 = 0	0	0	0	10	7	2	1	R = 0.80

r1 and R refer to resistant alleles for the sodium channel and *Ace-1* genes, respectively; s: susceptible allele.

Table 3. Toxicity of different plants extracts and insecticides against MED-Q1 and MED-Q3 biotypes from populations of *B. tabaci* collected in Burkina Faso

		Plant extracts						Chemical insecticides		
Type of oil/Chemical family		Seed oils			Essential oils			Pyrethroids	Neonicotinoids	Organophosphates
Plant/Insecticide name		*Lannea acida*	*Lannea microcarpa*	*Carapa procera*	*Ocimum americanum*	*Hyptis spicigera*	*Cymbopogon citratus*	Deltamethrin	Acetamiprid	Chlorpyrifos-ethyl
Biotype MED-Q1	Slope (±SE)	4.4 ± 1.2	3.6 ± 0.8	3.1 ± 0.7	1.3 ± 0.2	1.8 ± 0.3	1.3 ± 0.2	1.1 ± 0.3	0.98 ± 0.2	1.1 ± 0.3
	LC50(mg/liter)	6.9 (b)	5.9 (b)	4.7 (b)	1.3 (a)	0.67 (a)	1.5 (a)	42.6 (c)	121.1 (d)	12.5 (b)
	Confidence limits (95%)	5.2–8.3	4.6–6.5	3.4–5.3	0.8–1.7	0.54–0.75	1.1–1.9	36.8–53.0	109.7–147.2	8.0–17.1
Biotype MED-Q3	Slope (±SE)	2.5 ± 0.7	2.5 ± 0.6	2.9 ± 0.7	1.6 ± 0.3	1.3 ± 0.2	1.6 ± 0.3	1.3 ± 0.5	1.38 ± 0.4	1.2 ± 0.2
	LC_{50} (mg/liter)	2.4 (b)	2.5 (b)	2.6 (b)	0.44 (a)	0.53 (a)	0.6 (a)	21.4 (c)	25.6 (c)	3.7 (b)
	Confidence limits (95%)	1.6–3.3	1.7–3.5	1.3–3.3	0.2–0.6	0.35–0.66	0.43–0.71	16.4–32.6	15.9–34.8	1.4–6.7

For each biotype and each treatment, 540 individuals were tested. For each insecticide, LC_{50} values with the same letter are not significantly different. SE, standard error.

for MED-Q1 and 0.44 to 0.6 mg/liter for MED-Q3, but no significant difference was observed between essential oils for the two biotypes.

Discussion

In our samples, two biotypes MED-Q1 and MED-Q3, already observed in Burkina Faso, were found and both belong to the *B. tabaci* MED species (Gnankiné et al. 2013c). All the individuals collected on tomato belong to MED-Q1, whereas the ones sampled on *L. camara* belong to MED-Q3, a biotype that is mainly observed on this plant, while no other biotype has been found yet on *L. camara*. Interestingly, resistance alleles to pyrethroids have been found in the two biotypes at high frequency, but this study did not detect any mutation for the *ace1* gene that lead to organophosphate's resistance in MED-Q3 individuals while it is almost fixed in MED-Q1 population. It is known that *B. tabaci* biotypes have differing levels of insecticide resistance (Horowitz et al. 2005, Wang et al. 2010) and do not always share the same resistance mutation to a particular chemical class of insecticide (Alon et al. 2006, 2008).

In field populations of Burkina Faso, MED-Q3 individuals were more sensitive to insecticides than MED-Q1. This result can be linked to the fact that MED-Q3 seems to be restricted on *L. camara*, a plant that is not subjected to insecticide treatments. Indeed, Roush and Daly (1990) showed that the frequency of resistance genes to insecticides in pest populations is low when they are not exposed to any insecticide treatment, but once a given insecticide is widely used, the frequency of resistance genes increases. Thus, the resistance in the MED-Q1 population might be owing to the high selection pressure exerted on populations from vegetables crops. However, the low value observed for chlorpyrifos-ethyl (organophosphate) showed a relative efficiency and confirms that, now, this chemical is rarely used by farmers in *B. tabaci* control.

Anyway, the main point of this study is that we demonstrated that *B. tabaci* individuals are susceptible to plant extracts, essential or seed oils, whatever the biotype. Indeed, we showed that the essential oils of leaves of *Cymbopogon citratus*, *Ocimum americanum*, and *Hyptis spicigera* and the seed oils of *Lannea microcarpa*, *Lannea acida*, and *Carapa procera* were highly toxic for MED-Q1 and MED-Q3 adults. For all the plants tested, this susceptibility was higher than that of the two chemical insecticides tested (acetamiprid and deltamethrin). Previous studies already demonstrated the toxic, irritant, and repellent effect of some essential oils on *B. tabaci* individuals (Çalmaşur et al. 2006, Cloyd et al. 2009, Yang et al. 2010, Tia et al. 2013, Yarahmadi et al. 2013, Chae et al. 2014, Emilie et al. 2015, Deletre et al. 2016). There is no doubt that these insecticidal effects of essential oils are linked to their chemical composition. Chae et al. (2014) showed that essential oils extracted from lemon balm and summer savory contain oxygenated monoterpenes that are toxic against MEAM1 and MED-species of *B. tabaci*. Edson et al. (2015) also showed that *Pelargonium graveolens* essential oil and its associated oxygenated monoterpenes (geraniol and citronellol) were toxic against adult whiteflies. The high insecticidal effects of the essential oils of *O. americanum* could be associated to their high content of eucalyptol (41.4%), camphor (10.8%), and α-pinene (9.6%) for MED-Q3. The similar content was

found in rosemary oil, where eucalyptol (1-8-cineole) and camphor have been shown to be highly synergistic in their insecticidal action to the cabbage looper, an insect pest (Tak and Isman 2015).

The high insecticidal effects of the essential oils of *H. spicigera* could be attributed to their major compounds β-caryophyllene (22.15%), α-pinene (20.11%), and sabinene (10.26%) for MED-Q1. The similar contents was found in *H. spicigera* essential oil tested against *Sitophilus granarius* (L.) (Coleoptera: Dryophthoridae) and also exhibited high toxicity (Conti et al. 2011). Our future investigations will test the toxicity of the main contents of essential oils and also their combined effects. It is important to note that actually in United States, there are two essential oil-based commercial insecticides used against whitefly pests including *B. tabaci* produced by two firms. Requiem made by Bayer and Ecotrol[@] plus (rosemary oil, geraniol and peppermint oil) produced by KeyPlex.

However, to our knowledge, it is the first time that insecticidal activities of seed oils against *B. tabaci* were reported. Toxicity of seed oils seemed to be lower than the toxicity of essential oils, which could be explained by the nature and composition of these two types of oils. Essential oils are volatiles and have contact and fumigant toxicity (Yarahmadi et al. 2013, Chae et al. 2014, Deletre et al. 2016), whereas seed oils possess contact toxicity only. In addition, the essential oils are a complex mixture of several compounds which are known to possess insecticidal activities (Deletre et al. 2013). Seed oils are mainly composed of fatty acids which do not exhibit insecticidal activities. The biological properties of the seed oils are associated to minor components which in the most cases do not exceed 2% of the total lipid (Covas et al. 2006).

In conclusion, our results indicate that the seed oils and essential oils are more toxic than chemical insecticides for *B. tabaci* adults. Moreover, plant extracts have a reputation for being less hazardous to humans and other nontarget organisms and environmentally friendly nonpersistent bio-pesticides. The high frequencies of *Ace-1*^{R} and *kdr* alleles implied in organophosphate and pyrethroids, respectively, raise the question of the use of alternative control against this pest. Therefore, our results show that these plant extracts may represent an alternative strategy to the chemical use for controlling whitefly populations in Burkina Faso.

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