Research Article

The Essential Oils Compounds of *Lippia multiflora* Moldenke and *Cymbopogon schoenanthus* (L.) Spreng Repel and Affect the Survival of the Maize Pest *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Larvae

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Received 15 December 2023; Revised 27 February 2024; Accepted 13 March 2024; Published 1 April 2024

Academic Editor: Waheed Ali Panhwar

Plant-derived insecticides, such as essential oils, can be an effective alternative to replace synthetic chemical insecticides against *Spodoptera frugiperda*, which becomes increasingly resistant to synthetic products. This study aims to evaluate essential oils (EOs) effects on larval growth and development following feeding inhibition, growth regulation, and repellency of EOs of *Lippia multiflora* (Verbenaceae), *Cymbopogon schoenanthus* (Poaceae), and their combination. Topical application of EOs was used on *S. frugiperda* larvae for larvicidal effect or treating filter paper with the EOs to find repellency. The effect of EOs on food intake and larval growth was also evaluated. Several types of compounds have been identified in the EOs, mainly monoterpenes with the appearance of new compounds in the 1 : 1 combination. Bioassay results show that individuals and combinations of EOs significantly influenced *S. frugiperda* larval development. *L. multiflora* caused 100% mortality of L2 larvae within 24 hours at 3%. The Lm + Cs (1 : 1) EOs combination was the most effective with LC_{50} and LC_{90} of 1.02% and 1.92%, respectively. Lm + Cs (1/4 : 3/4) EOs caused the highest inhibition of food consumption, 0.0160 g consumed after food was treated with 2.2% concentration compared to food consumption of 0.0602 g for the control group at 24 hours. Lower food consumption caused the inhibition of larval growth and weight loss of 0.0005 g/day at the 2.2% EOs concentration. The highest repellency effect of the EOs was found in EOs of *L. multiflora*, exhibiting a repulsion of 83.33% of the larvae after 3 hours of exposure. This diversity in the biological actions of the EOs tested on *S. frugiperda* represents valuable options for contributing to integrated pest management and an alternative to synthetic chemical insecticides.

1. Introduction

In Burkina Faso, agricultural production of main cereals is dominated by maize and sorghum corps which are 1st and 2nd in production and consumption [1]. Despite the socioeconomic importance of maize in Burkina Faso, its cultivation faces several biotic and abiotic constraints. Among the abiotic constraints are poor soil, irregularity, and the poor spatiotemporal distribution of rainfall that strongly limits yields in the field [2]. In contrast, biotic constraints contribute to diseases and parasitic pressures [3].

Since 2017-2018, the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) [4], has caused very high damage in corn production [5]. The FAW is a polyphagous insect that eats 353 different plants with a marked preference for maize [6]. Infestations begin during the mid-to-late corn development stage. The FAW primarily eats corn husks. Occasionally, it also attacks ears and promotes the development of aflatoxin. Outbreaks of this pest were first reported in central and west Africa in 2016 by Goergen et al. [7]. Then, it was introduced to Burkina Faso during 2017-2018. Infestations of this caterpillar gradually spread themselves from 58,324 ha in 2017 to more than 94,108 ha in 2020 of infested crops, mainly maize [5]. Field infestations of FAW can lead to yield losses of 15–73% when 55–100% of plants are infested [8], which shows that this pest may be a serious threat to food security in sub-Saharan Africa.

To combat this pest, control methods are currently being implemented of inert substances (ash and sand), cultural, and physical controls, but the main control method remains synthetic insecticides. Farmers most often resort to chemical pesticides which can lead to human health risks [9] and cause environmental pollution and increased FAW resistance to insecticides [10]. Other biological control methods include the use of genetically modified varieties face regulatory, political, and consumer acceptance obstacles [11]. Furthermore, the increase in the use of chemical pesticides against this pest has led to pest resistance [12, 13].

Therefore, it is imperative to develop control mechanisms that are environment and human health friendly to biologically control *S. frugiperda* [14]. One of these methods is the use of botanical pesticides like essential oils (EOs). Plant-obtained EOs gained a lot of attention because they are natural phytoproducts with immense possibilities for public health usage. They can be extracted by steam distillation from different parts of the plant such as leaves, flowers, fruits, peels, and seeds [15, 16]. Plant EOs are complex mixtures of secondary metabolites including terpenes (monoterpenes, sesquiterpenes, and diterpenes), aromatic (phenylpropanoids), and aliphatic compounds with many functional groups [17, 18] with which resistance make them less likely to cause insecticide resistance because of diversity in their mode of action, allowing good insecticide resistance management.

Indeed, preliminary studies showed that the EOs from *Hyptis marrubioides* (Lamiaceae) ($LD_{50} = 18.49\ \mu g$/larva) and *Ocimum basilicum* (Lamiaceae) ($LD_{50} = 38.21\ \mu g$/larva) were toxic to FAW using topical bioassays [19]. In addition, the EOs of several plants have insecticidal and repellent activities against the larvae of this pest [20–25]. *Lippia multiflora* (Verbenaceae) and *O. americanum* (Lamiaceae) EOs contain diverse compounds showing potent larvicidal activity inhibiting larval growth and adult emergence [26]. These plants are used as insect repellents in Burkina Faso and are known for their insecticidal effects against other insects [27, 28]. The terpene compounds in these EOs inhibit larval and pupae developmental stages [19, 25, 26]. Indeed, certain phytopesticides already have shown larvicidal, ovicidal, antifeedant, and repellence activities against *S. frugiperda* [25, 29–31].

This report evaluates the larvicidal, antifeeding, growth-regulating, and repellent properties of *L. multiflora* and *Cymbopogon schoenanthus* (Poaceae) EOs that were used separately or in combination against *S. frugiperda* larvae.

2. Materials and Methods

2.1. Study Site. The study site is the "Laboratoire Central d'Entomologie Appliquée de Kamboinsé" (LCEAK) of the "Centre de Recherches Environnementales, Agricoles et de Formation de Kamboinsin" (CREAF-K). CREAF-K is one of the five research stations of "Institut de l'Environnement et de Recherches Agricole" (INERA) in Burkina Faso. Specifically, the study site is located in the province of Kadiogo, at the northeast exit of the city of Ouagadougou on the Ouagadougou-Kongoussi axis (latitude 12° 28 North, longitude 1° 32 West, and altitude 296 m).

2.2. Insect Rearing. The rearing of *S. frugiperda* was carried out in an insectarium with temperature of 26–30°C, with relative humidity of 60–80% and 12 : 12 h (light: dark) photoperiod according to Wangrawa et al. [26]. Larvae were placed in glass jars (1 L) and then daily fed with fresh maize leaves until pupation.

For late instar larvae, fresh-cut stems were also added to the maize leaves as an additional food source. The pupae were placed in cages until emergence. The newly emerged adults were fed with 10% honey and cotton wool soaked in water. Fresh maize leaves were obtained 30–50 days after seedlings (DAS) and placed in each cage for adult egg laying. The eggs were collected after oviposition and placed in jars for hatching. All subsequent generations were raised under the same conditions as described above avoiding contact with insecticides.

2.3. Extraction and Dilution of Essential Oils. The EOs of *C. schoenanthus* and *L. multiflora* leaves were used in this study. The taxonomic identification of the plants was made at the "Laboratoire de Biologie et d'Ecologie Végétales" (Université Jospeh KI-ZERBO, Burkina Faso), and the specimens were then kept at the university's information centre on biodiversity, called "infobio." Oil seeds extraction was done using hydrodistillation by a Clevenger apparatus at the "Institut de Recherche en Sciences Appliquées et Technologies" (IRSAT). The extracted oils were dissolved in absolute ethanol.

2.4. Chemical Identifications of Essential Oils. The major and minor chemical constituents of *L. multiflora* of *C. schoenanthus* EOs and their binary combination (1 : 1) were identified and quantified using gas chromatography-mass spectrometry (GC-MS) [28]. An aliquot (20 μL) of diluted solution (1/5000) was pipetted from each EO sample and placed into a vial with an insert (VWR, Radnor, PA) for use in the GC-MS (Trace 1310, Thermo Fisher Scientific) equipped with a 30 m column (I.D. 0.25 mm, #36096-1420, Thermo Fisher Scientific). Helium was used as the carrier gas at a constant flow of 1 cc/min. Samples were loaded into the GC/MS using an autosampler (TriPlus RSH, Thermo Fisher Scientific). The oven temperature was set at 45°C, held for 4 minutes followed by a heating gradient ramping to 230°C, and held for 6 minutes (run time: 28.5 min.). Chromatographed peaks were integrated manually using the Chromeleon software MS quantitative processing method (Thermo Fisher Scientific) and identified using the online NIST library (https://webbook.nist.gov/cgi/cbook.cgi?). Major peaks found with consistently high abundances across multiple samples for each EO were then recorded for comparison across species.

2.5. Topical Application of Essential Oils and Their Combination on L2 Larvae. The toxicity of the oils on L2 larvae was determined by topical application as described by Giongo et al. [32]. Toxicity was determined by testing five concentrations (1%, 1.6%, 2%, 2.4%, and 3%) of each EO and their combinations dissolved in absolute alcohol which was used to treat control groups. Larvae were placed in 15 cm diameter Petri dishes using a single larva per dish to avoid cannibalism. Each concentration corresponded to one treatment. Each treatment was repeated 3 times using 10 larvae (150 larvae of L2 per EO). Mortality was daily determined and the food was daily renewed for 3 days. Larvae that did not respond to the touch of flexible forceps were considered dead. Larval-rearing conditions are the same during topical application as described above.

2.6. Antifeeding Tests of Uniform-Size L3 Larvae. *Spodoptera frugiperda* L3 larval stage was chosen for these tests because from this stage onwards the feeding rate is significantly higher. Preliminary tests were carried out to determine the concentrations that cause strong and weak inhibitions of food intake. After that, for each EO and their combinations, five concentrations were tested (1%, 1.4%, 1.6%, 1.8%, and 2.2%). Pieces of corn husks of known mass 30–50 days after seedling were immersed for 5 seconds in EO solution corresponding to each treatment. Control group leaves were immersed in ethanol. The treated solutions on the maize husks were allowed to evaporate to dryness at room temperature for 10 minutes to avoid direct contact with the larva. The treated leaves were then offered to L3 larvae of *S. frugiperda* contained in Petri dishes. Each treatment was repeated ten times. Fifty larvae were used for each EO test [26].

During the evaluation of the feeding inhibition, larvae were monitored to assess other parameters such as survival time and mortality due to consumption of the treated substrate. The time taken (days) for a larva to live after consuming an oil-treated leaf was related to the concentration of the EOs used and to the mortality rate.

2.7. Larvae Growth Inhibition Test. Growth inhibition tests of L3 larvae carried out according to the Giongo et al. [33]'s method. Five concentrations of EO and their combinations (1%, 1.4%, 1.6%, 1.8%, and 2.2%) were used to treat pieces of 7 cm × 6 cm maize husks collected at 30–50 days after seedling (weight between 100 mg and 200 mg). The maize husks were immersed for 5 seconds in the EO test solution, and the solutions were allowed to dry at room temperature. Each treatment was repeated 10 times, and 50 larvae were used for each EO treatment.

Larval weights were daily determined for up to 11 days. At the end of the testing period, the growth rate (GR) was calculated by applying the following formula.

GR = $(A - B)/t$. A is the final mass of the larva, B the initial mass of the larva, and t is the duration of the test (days). A comparison was subsequently made between GR of the treated and GR of the corresponding controls.

2.8. Repellent Test. The EO repulsion test on L3 larvae of *S. frugiperda* was carried out using the preferential zone method on filter paper described by McDonald and Speirs [34]. Five concentrations (0.25%, 0.5%, 1%, 1.5%, and 2%) of each EO and their combinations were used. Absolute ethanol was used as a control treatment. Filter paper discs 9 cm in diameter were cut into 2 equal parts. For each EO and for each concentration, a volume of 1 ml was taken using a pipette and distributed evenly over one of the halves of the filter paper disc. No treatment was carried out on the other half disc of the filter papers. The treated half discs were then left to dry at room temperature for 5 minutes for the solvent to evaporate to avoid direct contact with the larva.

The two halves of discs (treated and untreated) were marked and anchored side by side using an adhesive strip serving as a transition zone inside a Petri dish. A piece of fresh maize leaf (used as bait) was placed in the centre of the treated half disc. Finally, an L3 stage larva of *S. frugiperda* was placed in the centre of the untreated disc.

The Petri dishes were closed and stored in the bio room. For each treatment, 20 repetitions were carried out. As a result, 600 larvae were used as part of this test which was carried out under the same conditions of humidity, light, and temperature as mentioned above.

Observations were carried out every hour for 3 hours. They consisted of counting for each concentration the number of larvae present on each half disk (treated and untreated). The larvae counted on the untreated parts were repelled from the treated halves. The average repellence percentage of each concentration was calculated according to the formula PR = $[(P_e - P_t)/100 - P_t] * 100$, where PR is

the percentage of repulsion, P_e is the percentage of larvae present on the untreated part, and P_t is the percentage of larvae present on the control part.

2.9. Data Analysis. The database was tested for normality (Shapiro test) and homogeneity (Bartlett test) of variances; these two conditions were first verified before choosing to use analyses of variance (ANOVA). The data on the EO application on L2 larvae and the amount of food consumed by the L3 larvae were assayed by ANOVA and non-parametric Kruskal–Wallis analysis. Significant ($P < 0.05$) determination was followed with pairwise. perm.t.test and dunnTest for two-by-two comparisons of means for ANOVA and the Kruskal–Wallis test, respectively, were performed. A Chi-square homogeneity test was used to follow the variation in the rate of the consumed food by L3 larvae minus the number of larvae that died due to the EO concentrations in the food. Significant $P < 0.05$ chisq. multcomp function was used to separate the means. R software version 4.0.2 (2020-06-22) and "RVAide-Memoire" [35] and FSA (simple fisheries stocks assessment methods) [36] packages was used for the statistical testing. Lethal concentrations of 50% and 90% (LC_{50} and LC_{90}) were determined by logistic regression using probit and XLStat software.

Kaplan–Meier estimation curves were used to visualize larval survival. They allowed us to compare the probabilities of survival of the larvae as a function of time among the different concentrations and the various EOs and their combinations.

3. Results

3.1. Essential Oils Composition. The analysis of the chemical composition of the essential oils (EOs) used allowed us to identify 55 compounds including 22 for *L. multiflora* (Lm), 21 for *C. schoenanthus* (Cs), and 26 for the combination of Lm + Cs (1 : 1) (Table 1). The total percentages of these compounds were 97.27%, 91.42%, and 81.09%, respectively, for *L. multiflora*, *C. schoenanthus*, and the Lm + Cs combination (Table 1). The majority compounds of *L. multiflora* were caryophyllene (27.66%), germacrene D (9.79%), P-cymene (8.19%), humulene (6.74%), and γ-terpinene (6.38%). The majority compounds of *C. schoenanthus* were elemol (22.78), (+)-4-carene (14%), β-elemene (8.61%), D-limonene (6.64%), and caryophyllene (6%). Isopiperitone (29.28%), caryophyllene (9.79%), (+)-4-carene (8.19%), P-cymene (6.74%), and β-elemol (6.84%) were the majority compounds contained in the combination of Lm + Cs (1 : 1). Furthermore, combination of EOs led to the formation of 11 new compounds including β-Thujene, α-Pinene, Cis-sabinene, piperitol, isopiperitone, carvacrol, thymol acetate, (E)-β-famesene, β-elemol, 10 -epi-γ-eudesmol, and 10-epi-β-eudesmol.

3.2. Larvicidal Effect of the Essential Oils on L2 Larvae. All the EOs tested by topical applications showed larvicidal activities towards the larvae of *S. frugiperda*, proportional to the applied concentrations and the exposure time (Table 2). The larvicidal activity of the EOs increased with the increase in the applied concentrations at 24 hours, 48 hours, and 72 hours after treatments. The analysis of variance of the mortality rate as a function of the concentration for each EO was highly significant ($P < 0.0001$) during each exposure time, and for each EO, the highest concentrations led to the highest mortality. Mortalities were also higher with increasing of the exposure time to the EOs. At 24 hours after topical application of EOs, the highest mortality rates were recorded at a concentration of 3%. These mortalities were 100%, 94.74%, 84.21%, 78.95%, and 100%, respectively, for the EOs of *L. multiflora* of *C. schoenanthus* and the combinations Lm + Cs (1/4 : 3/4), Lm + Cs (3/4 : 1/4), and Lm + Cs (1 : 1). *L. multiflora* caused total mortality of the larvae (100%) at 24 hours of exposure (Table 2).

The larvicidal activity of EOs persisted at 48 hours and 72 hours after larval treatments. At 48 hours after exposure, the cumulative mortality rate for each EO at a concentration of 3% was greater than 78.95%, and at 72 hours after topical applications, the cumulative mortality rate of each EO was greater than 84.21% when 3% of EOs was applied. The EOs of *L. multiflora*, *C. schoenanthus*, and Lm + Cs (1 : 1) caused total mortality (100%) of the larvae at 72 hours after topical application. Whereas the concentration of 2% Lm + Cs (1 : 1) caused total mortality of the larvae at 48 and 72 hours. The lowest lethality rate of EOs (21.05% ± 9.44) was recorded for treating *L. multiflora* at a concentration of 1% at 72 hours after exposure (Table 2).

The LC_{50} and LC_{90} show significant dependency for each EO treatment and the duration of the exposure time (Table 3). The larvicidal activity of the EOs persisted for three days after the topical applications. The highest larvicidal toxicity was found using the combination of Lm + Cs (1 : 1). Indeed, among the EOs tested, this treatment presented the highest lethal concentrations of LC_{50} and LC_{90}. Therefore, the Lm + Cs combination (1 : 1) is the most effective treatment using topical larvicidal treatments of the EOs tested. However, the Lm + Cs combination (3/4 : 1/4), compared to the other EOs tested, was the highest effective treatment with LC_{50} and LC_{90} at 24 h, 48 h, and 72 h of (LC_{50} (24 h) = 2.04% and LC_{90} (24 h) = 9.77%, LC_{50} (48 h) = 1.71% and LC_{90} (48 h) = 3.25%, and LC_{50} (72 h) = 1.40% and LC_{90} (72 h) = 2.85%) (Table 3).

3.3. Rate of Food Consumption of L3 Larvae. The quantities of food consumed by L3 stage larvae of *S. frugiperda* decreased with increasing EO concentrations and exposure time, regardless of the nature of the EO (Table 4). The reduction in the appetite of the tested larvae for preimmersed food in EOs (1%–2.2%, Table 4) is significant as compared with

Table 1: Chemical composition and % of essential oil from *Cymbopogon schoenanthus*, *L. Lippia multiflora*, and their (1 : 1) combination (news compounds in bold).

Retention time (min)	Compound	*C. schoenanthus* (%)	*L. multiflora* (%)	Lm + Cs (1 : 1) (%)
12.097	β-Thujene	—	—	**0.30**
12.247	α-Pinene	—	—	**0.12**
13.135	Cis-sabinene	—	—	**0.37**
13.342	β-Myrcene	0.30	2.38	0.42
13.675	α-Phellandrene	0.40	3.86	0.73
13.686	(+)-4-carene	14	—	11.53
13.781	3-Carene	0.30	—	—
13.924	α-Terpinene	—	—	**0.30**
14.083	P-cymene	0.70	8.19	8.85
14.128	D-limonene	6.40	1.54	1.15
14.189	Eucalyptol	1.20	5.34	1.69
14.274	Trans-β-ocimene	0.20	—	—
14.424	β-Ocimene	—	—	**0.40**
14.655	γ-Terpinene	—	6.38	1.74
15.345	Fenchone	0.30	—	—
15.373	Linalool	—	0.57	—
15.9	Trans-p-2.8-menthadien-1-ol	3.90	—	—
15.998	Trans-p-menth-2-enol	—	—	**0.33**
16.2	(+)-2-Bornanone	—	1.41	—
16.24	Camphor	—	—	**0.13**
16.825	Terpinen-4-ol	0.40	0.51	0.26
17.111	α-Terpineol	1.03	—	0.37
17.2	Trans-piperitol	1.32	—	—
17.406	Piperitol	—	—	**0.14**
18.124	Isopiperitone	—	—	**29.28**
18.4	Thymol	—	5.36	—
18.498	Carvacrol	0.40	—	4.38
19.321	Thymol acetate	—	—	**1.94**
19.532	Copaene	—	1.43	—
20.104	β-Elemene	8.61	—	—
20.212	Elemene	—	—	**1.17**
20.478	Caryophyllene	6	27.66	18.80
20.7	Beta-gurjunene	1.26	—	—
20.886	Cis-β-farnesene	—	3.07	—
20.964	Humulene	0.90	6.74	0.89
20.995	(E)-β-Famesene	—	—	**0.24**
21.311	Germacrene D	—	9.79	2.56
21.5	β-Selinene	2.88	—	—
21.5	(+-)-Germacrene D	—	—	—
21.7	γ-Muurolene	0.40	0.32	—
21.7	Bicyclogermacrene	—	—	—
21.8	β-Guaiene	1.80	0.50	—
22.185	Elemol	22.78	—	—
22.297	β-Elemol	—	—	**6.84**
22.723	Caryophyllene oxide	1.60	5.24	0.83
23.3	Cubenol	—	0.18	—
23.3	Cubenol	—	0.18	—
23.359	10-epi-γ-eudesmol	—	—	**0.71**
23.62	10-epi-β-eudesmol	—	—	**2.86**
23.7	α-Eudesmol	19.90	—	—
24.5	Geranyl-α-terpinene	—	0.16	—
26.1	α-Vetivol	—	0.27	—
26.8	m-Camphorene	—	0.33	—
27.2	p-Camphorene	—	0.19	—
27.3	1-Heptatriacotanol	0.29	—	—
	Total	**97.27**	**91.42**	**81.09**

Lm: *Lippia multiflora*; Cs: *Cymbopogon schoenanthus*. Compounds representing less than 0.1% on the same line in all essential oils have not been listed. Bold values represent values of new component find in the combination 1:1. Editor could remove Bold in total part in the table.

Table 2: Mortality rate of *Spodoptera frugiperda* larvae after 24 hours, 48 hours, and 72 hours of exposure to essential oils of *Cymbopogon schoenanthus*, *Lippia multiflora*, and their combination.

Treatment	*L. multiflora*	*C. schoenanthus*	Lm + Cs (3/4 : 1/4)	Lm + Cs (1/4 : 3/4)	Lm + Cs (1 : 1)
Mortality rate of larvae 24 hours after topical application of EOs (%)					
Absolute ethanol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1%	6.54 ± 5.26^{a}	36.84 ± 10.68^{b}	26.32 ± 9.99^{ab}	15.79 ± 8.72^{ab}	36.84 ± 10.68^{b}
1.6%	36.84 ± 10.68^{b}	57.89 ± 10.68^{b}	26.32 ± 9.99^{ab}	42.11 ± 10.84^{b}	52.63 ± 10.84^{b}
2%	36.84 ± 10.68^{b}	42.11 ± 10.84^{b}	47.37 ± 10.90^{bc}	42.11 ± 10.84^{b}	52.63 ± 10.84^{b}
2.4%	73.68 ± 9.44^{c}	57.89 ± 10.68^{b}	52.63 ± 10.84^{bc}	42.11 ± 10.84^{b}	63.16 ± 10.39^{b}
3%	100.00 ± 0.00^{d}	94.74 ± 4.75^{c}	78.95 ± 8.72^{c}	84.21 ± 7.78^{c}	94.74 ± 4.75^{c}
	$\chi^2 = 54.492$	$\chi^2 = 34.685$	$\chi^2 = 27.2$	$\chi^2 = 30.545$	$\chi^2 = 34.895$
Probabilities	df = 5	df = 5	df = 5	df = 5	df = 5
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Mortality rate of larvae 48 hours after topical application of EOs (%)					
Absolute ethanol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1%	15.79 ± 8.72^{a}	42.11 ± 10.84^{b}	26.32 ± 9.99^{ab}	15.79 ± 8.72^{a}	47.37 ± 10.90^{b}
1.6%	57.89 ± 10.68^{b}	63.16 ± 10.39^{ab}	42.11 ± 10.84^{bc}	57.89 ± 10.68^{b}	63.16 ± 10.39^{bc}
2%	63.16 ± 10.39^{b}	63.16 ± 10.39^{ab}	63.16 ± 10.39^{c}	68.42 ± 9.99^{b}	84.21 ± 7.78^{cd}
2.4%	84.21 ± 7.78^{bc}	78.95 ± 8.72^{c}	73.68 ± 9.44^{c}	78.95 ± 8.72^{b}	84.21 ± 7.78^{cd}
3%	100.00 ± 0.0^{c}	94.74 ± 4.75^{c}	78.95 ± 8.72^{c}	89.47 ± 6.54^{b}	100.00 ± 0.0^{d}
	$\chi^2 = 54.456$	$\chi^2 = 40.396$	$\chi^2 = 23.32$	$\chi^2 = 46.502$	$\chi^2 = 51$
Probabilities	df = 5	df = 5	df = 5	df = 5	df = 5
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Mortality rate of larvae 72 hours after topical application of EOs (%)					
Absolute ethanol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1%	21.05 ± 9.44^{a}	42.11 ± 10.84^{b}	31.58 ± 10.39^{b}	47.37 ± 10.90^{b}	57.89 ± 10.68^{b}
1.6%	57.89 ± 10.68^{b}	63.16 ± 10.39^{bc}	63.16 ± 10.39^{c}	68.42 ± 9.99^{bc}	63.16 ± 10.39^{b}
2%	78.95 ± 8.72^{bc}	68.42 ± 9.99^{bc}	85.21 ± 7.78^{c}	68.42 ± 9.99^{bc}	100.00 ± 0.0^{c}
2.4%	89.47 ± 6.54^{c}	84.21 ± 7.78^{bd}	73.68 ± 9.44^{c}	89.47 ± 6.54^{c}	94.74 ± 4.75^{c}
3%	100.00 ± 0.0^{c}	100.00 ± 0.0^{d}	84.21 ± 7.78^{c}	89.47 ± 6.54^{c}	100.0 ± 0.0^{c}
	$\chi^2 = 59.087$	$\chi^2 = 46.845$	$\chi^2 = 41.88$	$\chi^2 = 43.052$	$\chi^2 = 65$
Probabilities	df = 5	df = 5	df = 5	df = 5	df = 5
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Lm: *Lippia multiflora*; Cs: *Cymbopogon schoenanthus*; Lm + Cs (1 : 1): combination of Lm and Cs at 1 : 1 ratio; Lm + Cs (1/4 : 3/4): combination of Lm and Cs at the ratio 1/4 : 3/4 respectively; Lm + Cs (3/4 : 1/4): combination of Lm and Cs at the ratio 3/4 : 1/4, respectively. Mortality value of the same column for the same treatment followed identical alphabetic letters is not statistically different (Kruskal-Wallis test $P < 0.05$).

Table 3: Lethal concentrations 50 and 90 (LC_{50} and LC_{90}), their 95% confidence intervals, and regression parameters for *Spodoptera frugiperda* larvicidal activity (L2) of essential oils of *Lippia multiflora*, *Cymbopogon. schoenanthus*, and their combinations.

Essential oils	Exposure time (h)	LC_{50} (%)	CI_{95} (%)	LC_{90} (%)	CI_{95} (%)	*P*-value	Chi^2
L. multiflora		1.91	(0.35–2.23)	2.74	(2.47–3.71)	<0.0001	70.69
C. schoenanthus		1.71	(0.70–2.31)	3.14	(2.55–4.00)	<0.0001	39.40
Lm + Cs (1 : 1)	24	1.60	(0.34–2.11)	3.10	(2.63–4.12)	<0.0001	38.21
Lm + Cs (1/4 : 3/4)		2.07	(0.42–2.52)	3.70	(3.10–7.05)	<0.001	31.31
Lm + Cs (3/4 : 1/4)		2.04	(0.00–2.57)	3.77	(3.14–7.30)	<0.0001	29.27
L. multiflora		1.59	(1.01–1.92)	2.49	(2.14–2.89)	<0.0001	72.76
C. schoenanthus		1.43	(0.73–1.92)	2.66	(2.18–3.30)	<0.0001	50.28
Lm + Cs (1 : 1)	48	1.20	(0.43–1.58)	2.31	(1.97–2.79)	<0.0001	57.08
Lm + Cs (1/4 : 3/4)		1.57	(0.81–1.92)	2.80	(2.46–3.56)	<0.001	51.59
Lm + Cs (3/4 : 1/4)		1.71	(0.70–2.18)	3.25	(2.77–4.32)	<0.0001	36.87
L. multiflora		1.47	(0.93–1.79)	2.27	(1.96–2.66)	<0.0001	78.83
C. schoenanthus		1.37	(0.74–1.79)	2.42	(2.00–2.94)	<0.0001	60.49
Lm + Cs (1 : 1)	72	1.02	(0.33–1.35)	1.92	(1.63–2.32)	<0.0001	68.82
Lm + Cs (1/4 : 3/4)		1.25	(0.38–1.74)	2.62	(2.16–3.24)	<0.001	44.44
Lm + Cs (3/4 : 1/4)		1.40	(0.56–1.88)	2.85	(2.39–3.56)	<0.0001	41.58

LC: lethal concentration, CI: confident interval, Lm: *Lippia multiflora*; Cs: *Cymbopogon. schoenanthus*; Lm + Cs (1 : 1): combination of Lm and Cs at 1 : 1 ratio; Lm + Cs (1/4 : 3/4): combination of Lm and Cs at the ratio 1/4 : 3/4 respectively; Lm + Cs (3/4 : 1/4): combination of Lm and Cs at the ratio 3/4 : 1/4, respectively.

Table 4: Average masses of maize leaves treated with essential oils of *Lippia multiflora*, *Cymbopogon schoenanthus*, and their combinations consumed by L3 larvae of *Spodoptera frugiperda* after 12 and 24 hours.

Treatment	Average masses of substrate consumed (g)									
	0 to 12 h					12 to 24 h				
	L. multiflora	*C. schoenanthus*	Lm + Cs (3/4 : 1/4)	Lm + Cs (1/4 : 3/4)	Lm + Cs (1 : 1)	*L. multiflora*	*C. schoenanthus*	Lm + Cs (3/4 : 1/4)	Lm + Cs (1/4 : 3/4)	Lm + Cs (1 : 1)
Absolute ethanol	0.1182 ± 0.007^{a}	0.1182 ± 0.007^{a}	0.1182 ± 0.007^{a}	0.1182 ± 0.007^{a}	0.1182 ± 0.007^{a}	0.0602 ± 0.0040^{a}	0.0602 ± 0.0040^{a}	0.0602 ± 0.0040^{a}	0.0602 ± 0.0040^{a}	0.0602 ± 0.0040^{a}
1%	0.0979 ± 0.0048^{ab}	0.0843 ± 0.0093^{b}	0.0788 ± 0.0095^{b}	0.0701 ± 0.0043^{b}	0.0912 ± 0.0170^{b}	0.0497 ± 0.0065^{ab}	0.0428 ± 0.0047^{ab}	0.0328 ± 0.0064^{b}	0.0399 ± 0.0044^{b}	0.0461 ± 0.0116^{ab}
1.4%	0.0981 ± 0.0102^{ab}	0.0809 ± 0.0076^{b}	0.0880 ± 0.0106^{b}	0.0799 ± 0.0055^{b}	0.0704 ± 0.0084^{b}	0.0482 ± 0.0051^{ab}	0.0459 ± 0.0072^{ab}	0.0278 ± 0.0067^{b}	0.0232 ± 0.0028^{bc}	0.0541 ± 0.0053^{ab}
1.6%	0.0901 ± 0.0089^{ab}	0.0780 ± 0.0071^{b}	0.0592 ± 0.0078^{b}	0.0692 ± 0.0023^{b}	0.0707 ± 0.0055^{b}	0.0462 ± 0.0058^{ab}	0.0373 ± 0.0047^{b}	0.0365 ± 0.0074^{b}	0.0273 ± 0.0059^{bc}	0.0333 ± 0.0065^{b}
1.8%	0.0871 ± 0.0060^{b}	0.0725 ± 0.0087^{b}	0.0891 ± 0.0013^{b}	0.0747 ± 0.0054^{b}	0.0606 ± 0.0080^{b}	0.0456 ± 0.0046^{ab}	0.0346 ± 0.0083^{b}	0.0219 ± 0.0063^{b}	0.0289 ± 0.0048^{bc}	0.0420 ± 0.0047^{ab}
2.2%	0.0608 ± 0.0074^{c}	0.0734 ± 0.0072^{b}	0.0671 ± 0.0093^{b}	$\pm 0.08860.0106^{b}$	0.0536 ± 0.0047^{b}	0.0259 ± 0.0094^{b}	0.0240 ± 0.0049^{b}	0.0291 ± 0.0042^{b}	0.0160 ± 0.0050^{c}	0.0136 ± 0.0040^{c}
Probabilities	$F_{5;53} = 6.11$ $P = 0.0001$	$\chi^2 = 19.164$ df = 5 $P = 0.0018$	$\chi^2 = 27.589$ df = 5 $P < 0.0001$	$F_{5;53} = 10.04$ $P < 0.0001$	$\chi^2 = 27.855$ df = 5 $P < 0.0001$	$F_{5;53} = 2.46$ $P = 0.0445$	$F_{5;53} = 4.35$ $P = 0.0021$	$\chi^2 = 19.164$ df = 5 $P = 0.0013$	$F_{5;53} = 11.57$ $P < 0.0001$	$\chi^2 = 19.164$ df = 5 $P < 0.0001$

LC: lethal concentration, CI: confident interval, Lm: *Lippia multiflora*;Cs: *Cymbopogon schoenanthus*; Lm + Cs (1 : 1): combination of Lm and Cs at 1 : 1 ratio; Lm + Cs (1/4 : 3/4): combination of Lm and Cs at the ratio 1/4 : 3/4, respectively; Lm + Cs (3/4 : 1/4): combination of Lm and Cs at the ratio 3/4 : 1/4, respectively. Means ± standard errors having the same superscript lowercase letters in the same column are not significantly different (linear ANOVA and Kruskal–Wallis test, $\alpha = 0.05$).

consumption of food that was not immersed in EOs ($P < 0.05$). The quantity of food consumed after the first 12 hours (0–12 hours) was larger than food consumed after 24 hours (12–24 hours) (Table 4).

After 12 hours of exposure, the EO of *L. multiflora* showed the lowest food consumption or the lowest appetite, and quantities consumption of food after immersion in EOs varied between 0.0979 g and 0.0608 g, respectively, for different concentration (1%–2.2%) (Table 4). The quantity of the food consumed was lower than that of the food treated with alcohol (control) (0.1182 g). The combinations caused almost the same inhibition of food consumption (average food weight and EOs concentrations) (Table 4). The amount of food consumed varied between 0.0912 g and 0.536 g, respectively, after treatment with 1%–2.2% EO. In general, at 24 hours after food treatment with EOs, the Lm + Cs (1/4 : 3/4) and Lm + Cs (3/4 : 1/4) combinations showed the best inhibition of food consumption by larvae of *S. frugiperda*. The amount of food consumed was between 0.309 g and 0.016 g for concentrations of 1%–2.2% of EO, respectively. However, compared to other EOs tested, the *L. multiflora* EOs caused weak inhibition of larval food consumption and the quantity of food that was eaten by the larvae was the highest after 24 hours (0.0497 g and 0.259 g) after EO treatment (1%–2.2%, respectively) (Table 4). Moreover, these amounts of food consumed were still much lower than compared with controls that were treated only with alcohol (0.0602 g, Table 4). Treatments with combinations of 2 different EOs were much more effective than a single EO treatment.

3.4. Effect of Essential Oils on the Growth of S. frugiperda L3 Larvae. In all cases, feeding of *S. frugiperda* L3 larvae with EOs-treated food led to reduction of the daily weight gain of *S. frugiperda* larvae (Table 5). The reduction in daily weight gain is highly significant ($P < 0.0001$) for all the EOs that were tested. Only L. *multiflora* EO did not show negative daily weight gain at 2.2%. The other EOs and combinations at the same concentration induced a negative daily weight gain (Table 5). The larval weight loss of feeding food treated with the Lm + Cs (1/4 : 3/4) combination attained the best inhibition of larval growth and the lowest daily weight gains of 0.0149 g/day–0.0005 g/day for concentrations of 1%–2.2% of EO, respectively, and the strongest growth inhibition (Table 5). *L. multiflora* EOs showed the lowest inhibition of larval growth and the highest daily weight gain. This weight gain varied between 0.0184 g/day (at the concentration of 1% EO) and 0.0126 g/day (at the concentration of 2.2% EO) (Table 5).

3.5. Effect of Essential Oils on the Mortality of S. frugiperda Larvae after Food Ingestion. Larval mortality was followed feeding larvae with presoaked food at increasing EOs concentrations. The results show that dietary intake of EOs treated food causes mortality to *S. frugiperda* larvae and the mortality depends of EOs, combinations, and concentrations (Figure 1). The mortality rate is significant at EOs concentrations of 1.4%–2.2% ($P < 0.05$) except for *C. schoenanthus*. At 1.8%–2.2%, larval mortality was 100% for all EOs combinations. Using individual EOs, only *L. multiflora* EOs caused the same larval mortality rate. The lowest mortality rate was 30% at 1% concentration of *C. schoenanthus* EO and the combination of Lm + Cs (3/4 : 1/4) and Lm + Cs (1 : 1). These rates were all higher than that of the control treatment (20%) except at 1% for Lm + Cs (3/4 : 1/4) and Lm + Cs (1 : 1) (Figure 1).

3.6. Survival Analysis. The survival analysis shows that all EOs exhibit a dose-dependent effect on the *S. frugiperda* larval survival (Figure 2(a)). For all the EOs tested, at a concentration of 1%, the longevity of the larvae is not significantly different from that of the control treatment. However, the EOs of *C. schoenanthus* (at concentrations of 1%, 1.4%, and 1.6%) and the Lm + Cs mixture (1 : 1) (at a concentration of 1%) extended larval survival average longevity (8.9 and 10.9 days, respectively) as compared with the control group (8.5 days). On the other hand, at a dose of 2.2% of all tested EOs and combinations, the longevity of the larvae did not exceed 2 days (Figure 2(c)).

Furthermore, the Lm + Cs (3/4 : 1/4) combination is the best treatment, causing a significant reduction in the longevity of *S. frugiperda* larvae (Figure 2(b)). The average longevity for this EOs was between 1 day and 7 days. *C. schoenanthus*, on the other hand, was not effective in reducing the longevity of *S. frugiperda* larvae as compared with the other EOs.

3.7. Rate of Pupal Emergence. EOs and their combinations reduced the rate of pupal emergence after treatment with EOs at concentration of 1.4%–2.2% (Table 6). The concentration factor analysis of each EO shows that this variation in the rate of pupal emergence is statistically significant ($P < 0.05$). The drop in the rate of pupal emergence is high at 1.4% concentration for all EOs tested and for the combinations of Lm + Cs (3/4 : 1/4) and Lm + Cs (1 : 1). No pupal emergence was observed at concentrations of 1.4%, 1.6%, and 1.8% of *L. multiflora* EOs and for *C. schoenanthus*, Lm + Cs (3/4 : 1/4) and Lm + Cs (1/4 : 3/4) combination at concentration of 2.2% (Table 6).

3.8. Repellent Activities of Essential Oils. The EOs of *L. multiflora* and *C. schoenanthus* and their combinations exhibited repelled *S. frugiperda* larvae when tested for three hours but treating with Lm + Cs (3/4 : 1/4) weakly repelled *S. frugiperda* larvae (Figures 3(b) and 3(c) for Lm + Cs (1/4 : 3/4)).

The repellent effects of EOs are dose dependent. The concentration effect tested by ANOVA for each EO and their combinations indicates a highly significant difference ($P < 0.001$) between the EO concentrations that were used, indicating that the separate EO and the Lm + Cs (1 : 1) mixture in higher concentrations caused more repellent than the other EO combinations.

Table 5: Effect of essential oils from *Lippia multiflora*, *Cymbopogon schoenanthus*, and their combinations on the growth of *Spodoptera frugiperda* L3 larvae.

Treatment	Average daily weight gain of larvae (g/day)				
	L. multiflora	*C. schoenanthus*	Lm + Cs (3/4 : 1/4)	Lm + Cs (1/4 : 3/4)	Lm + Cs (1 : 1)
Absolute ethanol	0.0172 ± 0.0010^{a}	0.0172 ± 0.0010^{ab}	0.0172 ± 0.0010^{a}	0.0172 ± 0.0010^{a}	0.0172 ± 0.0010^{a}
1%	0.0184 ± 0.0012^{a}	0.0209 ± 0.0012^{a}	0.0160 ± 0.0013^{a}	0.0149 ± 0.0012^{a}	0.0190 ± 0.0011^{a}
1.4%	0.0143 ± 0.0016^{a}	0.0158 ± 0.0012^{bc}	0.0076 ± 0.0010^{bc}	0.0086 ± 0.0011^{bc}	0.0132 ± 0.0018^{a}
1.6%	0.0147 ± 0.0019^{a}	0.0135 ± 0.0012^{bc}	0.0101 ± 0.0008^{b}	0.0070 ± 0.0010^{b}	0.0136 ± 0.0016^{a}
1.8%	0.0079 ± 0.0019^{b}	0.0115 ± 0.0013^{c}	0.0036 ± 0.0014^{c}	0.0041 ± 0.0008^{c}	0.0152 ± 0.0024^{a}
2.2%	0.0126 ± 0.002^{a}	-0.0022 ± 0.0009^{d}	-0.0032 ± 0.0019^{d}	-0.0005 ± 0.0008^{d}	-0.0052 ± 0.0012^{b}
Probabilities	$\chi^2 = 30.88$ df = 5 $P < 0.0001$	$\chi^2 = 85.811$ df = 5 $P < 0.0001$	$\chi^2 = 104.66$ df = 5 $P < 0.0001$	$\chi^2 = 119$ df = 5 $P < 0.0001$	$\chi^2 = 45.721$ df = 5 $P < 0.0001$

LC: lethal concentration, CI: confident interval, Lm: *Lippia multiflora*; Cs: *Cymbopogon schoenanthus*; Lm + Cs (1 : 1): combination of Lm and Cs at 1 : 1 ratio; Lm + Cs (1/4 : 3/4): combination of Lm and Cs at the ratio 1/4 : 3/4, respectively; Lm + Cs (3/4 : 1/4): combination of Lm and Cs at the ratio 3/4 : 1/4, respectively. Means ± standard errors having the same superscript lowercase letters in the same column are not significantly different (linear ANOVA and Kruskal–Wallis test, $\alpha = 0.05$).

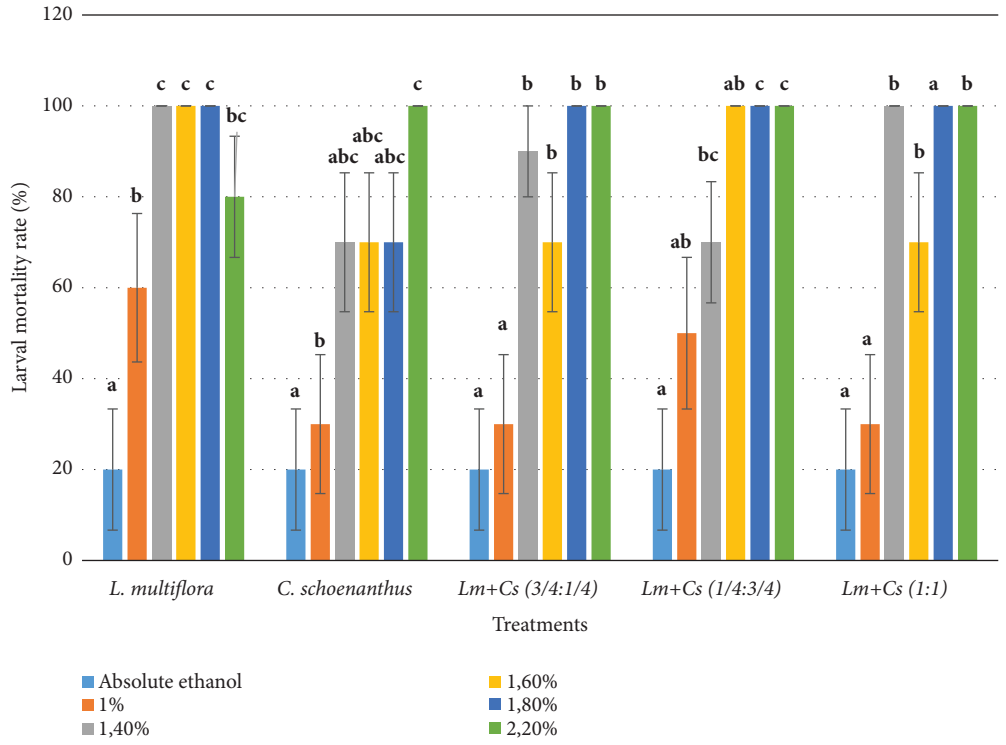
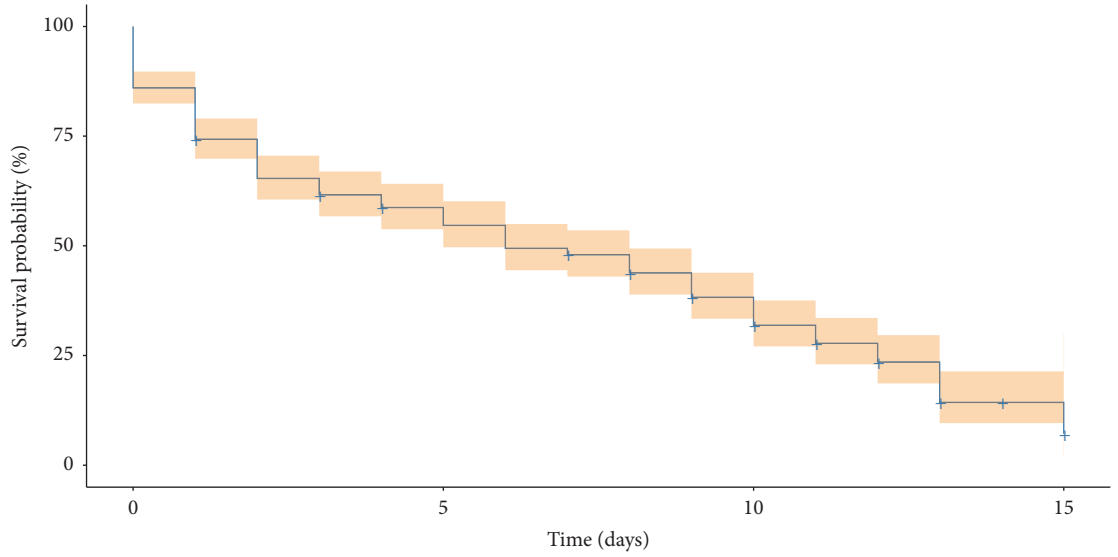
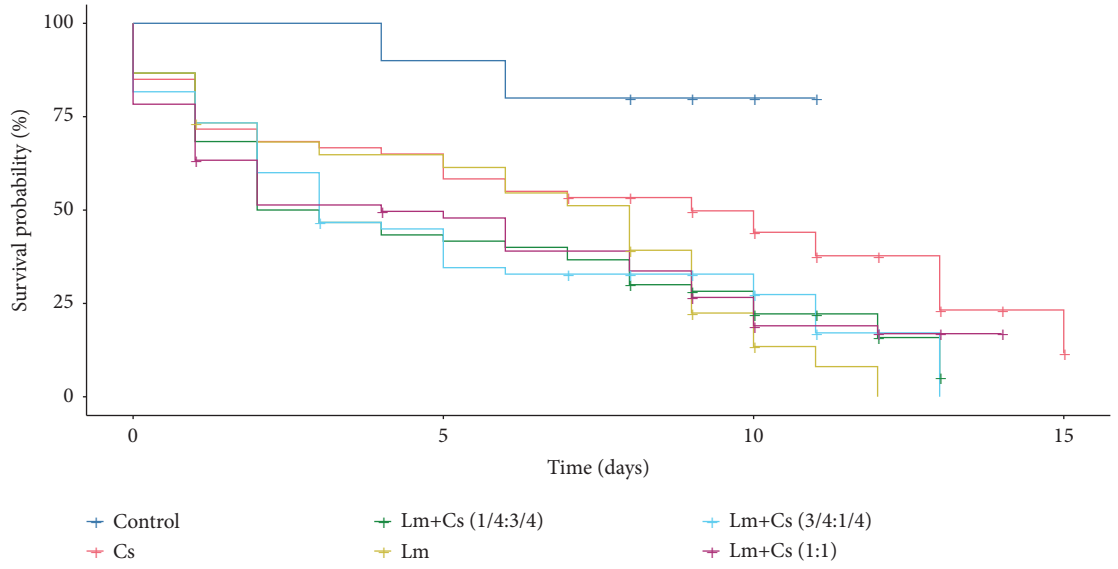


Figure 1: Larval mortality rate of *Spodoptera frugiperda* due to food intake of maize leaves treated with essential oils of *Lippia multiflora*, *Cymbopogon schoenanthus*, and their combinations.

The EOs that were used at a concentration of 0.5% repelled more than 50% of the L3 larvae that were tested after 1 hour of exposure (Figure 3(a)). The highest repellent rate of 93.75% ± 5 was obtained with the EO of *C. schoenanthus* at a concentration of 2.2%. Furthermore, among the EO solutions tested, the lowest repulsive rate (25% ± 11.24) was obtained with the Lm + Cs (1/4 : 3/4) combination at 0.25% concentration.



(a)



(b)

Figure 2: Continued.

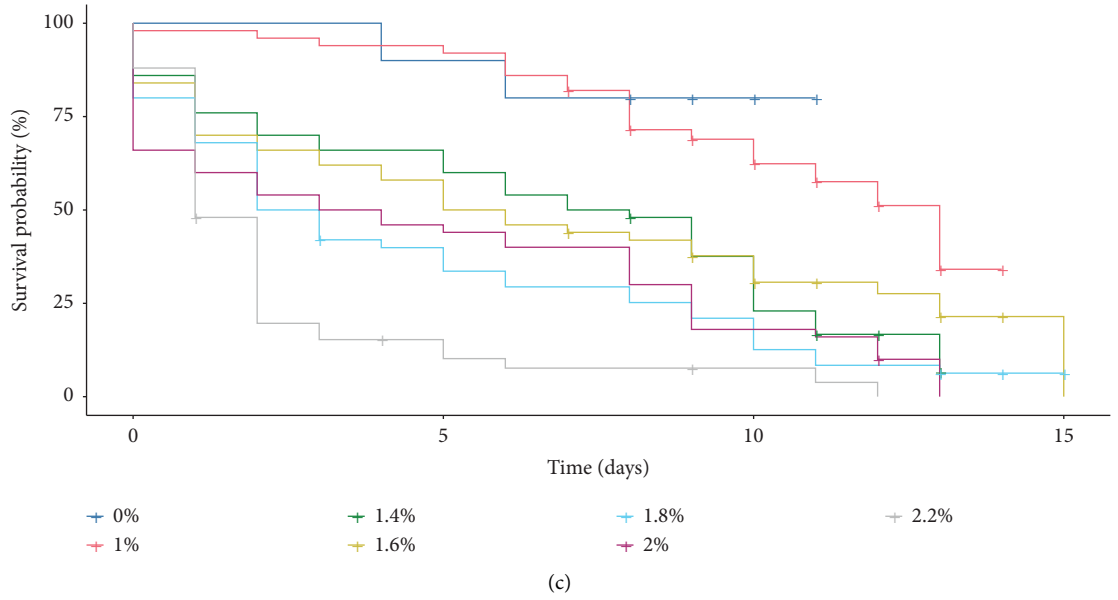


Figure 2: Cumulative survival curves of L3 larvae of *Spodoptera frugiperda* after ingestion of maize leaves treated with essential oils of *Lippia multiflora, Cymbopogon schoenanthus*, and their combinations. (a) General trend of larval survival with all treatments for 15 days; (b) general trend of larval survival with each treatment for 15 days, and (c) general trend of larval survival with each concentration for 15 days.

Table 6: Rate of *Spodoptera frugiperda* pupal emergence after feeding maize leaves treated with essential oils of *Lippia multiflora, Cymbopogon. schoenanthus*, and their combinations.

Treatment	Rate of pupae formed				
	L. multiflora	*C. schoenanthus*	Lm + Cs (3/4 : 1/4)	Lm + Cs (1/4 : 3/4)	Lm + Cs (1 : 1)
Absolute ethanol	80.00 ± 13.33^{a}	80.00 ± 13.33^{a}	80.00 ± 13.33^{a}	80.00 ± 13.33^{a}	80.00 ± 13.33^{a}
1%	40.00 ± 16.33^{b}	70.00 ± 15.28^{a}	70.00 ± 15.28^{a}	50.00 ± 16.67^{ab}	70.00 ± 15.28^{a}
1.4%	0.00 ± 0.00^{b}	30.00 ± 15.28^{ab}	10.00 ± 10.00^{b}	30.00 ± 13.33^{bc}	0.00 ± 0.00^{b}
1.6%	0.00 ± 0.00^{b}	30.00 ± 15.28^{ab}	30.00 ± 15.28^{b}	20.00 ± 15.28^{bc}	30.00 ± 15.28^{b}
1.8%	0.00 ± 0.00^{b}	30.00 ± 15.28^{ab}	10.00 ± 10.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
2.2%	20.00 ± 13.33^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	20.00 ± 13.33^{b}
Probabilities	$\chi^2 = 28.217$ df = 5 $P < 0.0001$	$\chi^2 = 18.028$ df = 5 $P < 0.0029$	$\chi^2 = 25.37$ df = 5 $P = 0.0001$	$\chi^2 = 22.476$ df = 5 $P = 0.0004$	$\chi^2 = 26.255$ df = 5 $P < 0.0001$

LC: lethal concentration, CI: confident interval, Lm: *Lippia multiflora*; Cs: *Cymbopogon schoenanthus*; Lm + Cs (1 : 1): combination of Lm and Cs at 1 : 1 ratio; Lm + Cs (1/4 : 3/4): combination of Lm and Cs at the ratio 1/4 : 3/4, respectively; Lm + Cs (3/4 : 1/4): combination of Lm and Cs at the ratio 3/4 : 1/4, respectively. Means ± standard errors having the same superscript lowercase letters in the same column are not significantly different (linear ANOVA and Kruskal–Wallis test, $\alpha = 0.05$).

After two hours of exposure, the EOs and their combinations repelled larval *S. frugiperda* (Figure 3(b)). The repulsive rates, however, obtained at this period are lower as compared to the rates obtained 1 hour after exposure for all treatments. The repellence rates of this EO were between 40% for the concentration of 0.25% and 85% for the concentration of 2%, whereas the Lm + Cs (1/4 : 3/4) and Lm + Cs (3/4 : 1/4) combinations showed the lowest repulsive rate against *S. frugiperda* larvae.

The EOs tested repelled larvae after 3 hours of exposure (Figure 3(c)). However, the rates observed are slightly lower than those observed at 1 hour and 2 hours of exposure. After 3 hours of exposure, *L. multiflora* EO presented the highest repellent rates compared to the other EOs tested.

The representative curves of each EO as a function of exposure time show that the repulsive power of EOs is reduced with an increase in exposure time (Figure 3(d)). Indeed, the average repulsive rates of the EOs were between 56.25% and 66.25% after one hour of exposure. But after three hours of exposure, the average repellence rates of the EOs were between 40% and 54.74%.

4. Discussion

The analysis of the chemical composition of EOs from *L. multiflora*, *C. schoenanthus*, and their combination (1 : 1) show the presence of several types of terpene compounds. Previous studies reported the composition of *L. multiflora*

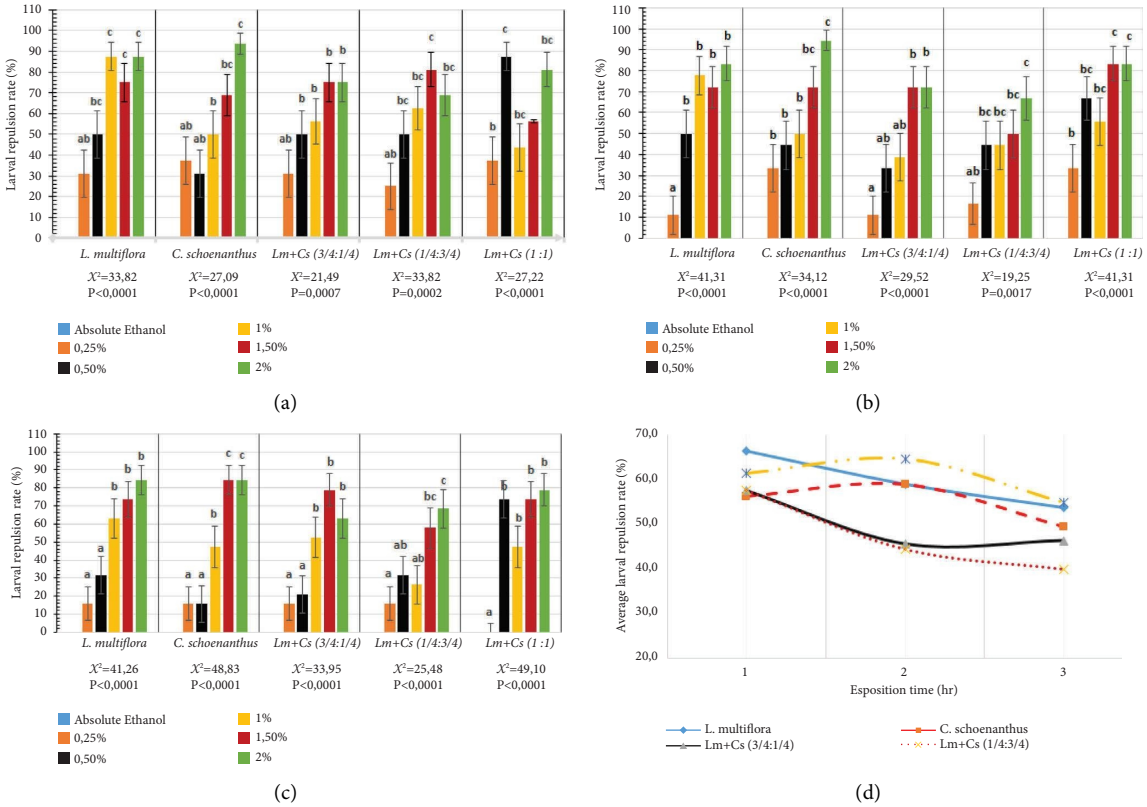


FIGURE 3: Repellent effect of essential oils from *Lippia multiflora*, *Cymbopogon schoenanthus*, and their combinations on *Spodoptera frugiperda* L3 larvae: (a) repellent effect after one hour of exposure; (b) repellent effect after two hours of exposure; and (c) repellent effect after three hours of exposure. (d) The general trend of the average repellent rate of essential oils and their combinations as a function of exposure time.

oils [37, 38], *C. schoenanthus* [39, 40], and the combination of these two EOs [28, 41]. *Lippia multitiflora* oil is mainly composed of caryophyllene, germacrene D, P-cymene; humulene, and γ-terpinene as reported by studies in Burkina Faso and Ivory Coast [37, 38, 42]. The EO of *C. schoenanthus* was mainly composed of elemol, (+)-4-carene, β-elemene, D-limonene, and caryophyllene. The reports by Alitonou et al. [43] of Aous et al. [44] and Hashim et al. [39] confirmed the presence of these compounds as the major components of the *C. schoenanthus* EO. The combination of these two EOs is mainly composed of isopiperitone, caryophyllene, and (+)-4-carene. New compounds, previously absent in EO, appear in the combination including β-thujene, α-pinene, Cis-sabinene, piperitol, isopiperitone, carvacrol, thymol acetate, (E)-β-famesene, β-elemol, 10 -epi-γ-eudesmol, and 10-epi-β-eudesmol probably due to components interaction. The differences in EO composition and yield of these two plants would be linked to intrinsic factors (subspecies, age, and organ) or extrinsic factors (climate, growing conditions, or extraction methods) [44, 45].

All the EOs in all combinations and concentrations that were tested showed larvicidal activities against L2 larvae of *S. frugiperda*. Mortality rates varied and depended on the concentration that were applied and on exposure time after the topical application. *L. multiflora*, *C. schoenanthus*, and the combination of Lm + Cs (1 : 1) showed the greatest efficacy against *S. frugiperda* larvae after 72 h. These oils and this combination, respectively, contained caryophyllene, germacrene D, P-cymene, β-elemol, caryophyllene, (+)-4-carene, P-cymene, isopiperitone, and β-elemol as the majority compound. The larvicidal activity in the EO could be linked to the terpene compounds present at high and low concentrations in each EO. The larvicidal activities of the terpenes have been reported before [46, 47] and previous studies specified the lethal effect of the EOs from *Piper corcovadensis* (Piperaceae), *Piper marginatum* (Piperaceae), *Piper arboretum* (Piperaceae), *C. schoenanthus*, *L. multiflora*, and *O. americanum* against *S. frugiperda* larvae [20, 26, 48–50]. The differences in mortality are probably due to the differences in the composition of each EO.

Lippia multiflora and *C. schoenanthus* caused 100% mortality of the treated larvae at 24 and 48 hours, respectively, with a concentration of 3% EO. These results are in agreement with those of Ketoh et al. [51], who reported that the EO of *C. schoenanthus* and its main constituent, piperitone, had insecticidal activity against neonate larvae of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae). Likewise, limonene, one of the main compounds in *C. schoenanthus* oil, found in certain citrus fruits, acts as a nerve toxin and contact poison [52]. EOs of *C. schoenanthus* are toxic against *Lycoriella ingenua* (D.) (Diptera:

Sciaridae) larvae [53]. These results are also in agreement with those of Negrini et al. [22], who observed a mortality of 92% after topical application of *Lippia microphylla* (Verbenaceae) EO at a concentration of 15 mg/g to *S. frugiperda*, showing a potential for the *L.* microphylla in *S. frugiperda* control [22].

Between combinations, the Lm + Cs (1 : 1) EOs combination was the most effective depending on the concentration applied and the mortality rate that was associated with the various concentrations. This combination presented the lowest LC_{50} and LC_{90} concentrations throughout the experimental period. The effectiveness of this EO would probably be linked to a synergistic effect between the components of the two oils causing lethal toxicity to the *S. frugiperda* larvae. Previous research on mixtures of these two oils reported this synergy of lethal toxicity against adult *C. maculatus* [41] and larvae of *Anopheles funestus* (Diptera: Culicidae) and *Culex quinquefasciatus* (Diptera: Culicidae) [28]. In addition, the combination of the two EOs led to the formation of another major compound, isopiperitone (29.28%), that was absent in the two EO taken separately but appeared after mixing the two EOs and is known for its insecticidal activity against cowpea bruchids [51]. This majority compound could act synergistically with the other new and old compounds to manifest the lethal effect against *S. frugiperda* larvae.

On the other hand, the combination of Lm + Cs (3/4 : 1/4) and Lm + Cs (1/4 : 3/4) was less effective as compared with the two oils applied individually to the larvae according to the strong CL_{50} and CL_{90} observed after 24 hours of exposure to treatment. This low efficiency would probably be linked to an antagonistic effect exerted by several compounds that are found in the EOs [28]. Results different than what we found were reported by Deletre et al. [54] on mixtures of *Thymus vulgaris* (Lamiaceae) and *Cinnamomum zeylanicum* (Lauraceae) oils of 3/4 : 1/4 and 1/4 : 3/4, respectively. These EO combinations were synergistically amplifying their toxicities against *Anopheles gambiae* (Diptera: Culicidae), and these differences may indicate that larval mosquitoes and larval lepidopterans may have different sensitivities.

All EO combinations and single applications to the food caused a reduction in the food intake of L3 larvae of *S. frugiperda*. The reduction in food intake was significant with increasing concentration for all EOs and combinations. The EO combinations presented the best inhibition of larval food intake. The reduction of food intake would indicate that the EO may have larval antifeeding properties which affect the insect's nervous system [55]. Akhtar et al. [55] reported that an insect's species-specific response as to whether to feed or not to feed depends on how the chemical interaction between all the constituents of a mixture is detected by the taste sensilla. Furthermore, our results are consistent with those of Munoz et al. [56] who showed that methanol and acetate extracts of *Calceolaria talcana* (Calceolariaceae) which also contain terpenes [57] induce *Drosophila melanogaster* (Diptera: Drosophilidae) larval antifeeding effect on and in *S. frugiperda*. Similarly, the artificial diet treated with the EO of *Cymbopogon flexuosus* (Verbenaceae) leads to a reduction in consumption and mortality of FAW with an LT_{50} of 18.85 h [23]. Several other studies confirmed the antifeeding effect of EOs on insects of the Spodoptera genus [58–60]. Several other studies confirmed the antifeeding effect of EOs from *Lippia* alba (Verbenacea) and *Callistemon lanceolatus* (Sm.) (Myrtaceae) against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) [61] and larval *Rhipicephalus appendiculatus* (Ixodida: Ixodidae) [62].

The antifeeding affected normal larval growth, causing a progressive decrease in the daily weight gain. EOs of all the plants at a concentration of 2.2% caused 100% inhibition of larval growth, and the affected larvae became anorexic and died. This effect of the EOs on larval growth is probably linked to the deterrent and antifeeding properties of the EOs that were tested. Our results are similar to those reported by Giongo et al. [32] who showed that molecules such as scopoletin isolated from the branches of *Trichilia pallens* (Meliaceae) and triglycerides isolated from the branches, fruits, and stems of *Trichilia ciliata* (Meliaceae) caused a 23% and 24% reduction in the larval weight of *S. frugiperda*, respectively.

In addition, diterpene from *C. talcana* in the diet of larval *D. melanogaster* and *S. frugiperda* caused growth inhibition [57]. The reduction in larval growth by ingestion of the treated food resulted in larval mortality which would partly be due to the toxicity of the EOs in the digestive tract of the larvae [17] and also because not enough food was eaten.

The EOs of *L. multiflora* and combination Lm + Cs (1 : 1) that we used in this study significantly reduced the pupal emergence rate of the tested larvae at low concentrations. The other treatment also reduced pupal emergence but at higher concentrations. The reduction in the pupal emergence rate would be linked to the inhibition of food uptake; therefore, a critical weight that is necessary for pupation and adult emergence was not achieved and, therefore, pupation was inhibited and hence mortality was enhanced.

At high concentrations (1.8% and 2.2%), all oils and oil combinations lead to a significant reduction in larval longevity. *Lippia multiflora* EO and Lm + Cs (3/4 : 1/4) were the best and most effective treatment while *C. schoenanthus* EOs were worst in reducing longevity. Larval longevity was short (1 day) at high EOs concentration (2.2%) as compared with control groups that were much longer (8.5 days). The short life span is due to the lack of eating and the inherent toxicity of the EOs. These results agree with those of Oliveira et al. [23] who had shown that EOs of *Cymbopogon flexuosus* (Poaceae) reduced growth, increased mortality, and shortened the development time of *S. frugiperda*.

Similarly, Zemenzer [63] showed that the EOs of *Rosmarinus officinalis* (*Lamiaceae*) also reduced the longevity of adult *Tribolium castaneum* (Coleoptera: Tenebrionidae).

It is interesting to note that the EOs of *L. multiflora* and *C. schoenanthus* as well as their combinations exhibited repellence concentration-dependent activity against larval *S. frugiperda*, possibly linked to the strong odour emitted by the terpenes in the EOs of these plants. The odours emitted by the terpenes also deter the larvae from wandering and feeding on treated plants as was reported by Bokobana et al. [64] that showed that *C. schoenanthus* EOs exhibited

a concentration-dependent repellence effect on *Aphis gossypii* Glover (Aphidoidea), a cotton pest. These authors reported that piperitone, one of the major constituents of the EO, is responsible for this repellent effect. Several previous studies also showed the repellent effects of EOs against insect pests [65–67].

Our study also shows that the EOs lost their effectiveness over time, especially when low concentrations of EOs (0.25% and 0.5%) were used. The loss of activity is probably linked to the volatility of several EOs and this was also reported by Aissaoui et al. [45] who indicated that the EOs of *R. officinalis* dissipate after 24 h of exposure to the air.

5. Conclusion

This study shows that plant's EOs are mainly terpene. Mixing of two EOs enhanced the formation of new terpene compounds, which were mostly absent or marginally present in the composition of the two oils that were extracted separately. The EOs of *L. multiflora* and *C. schoenanthus* and their combinations have topical larvicidal activities on *S. frugiperda*. EOs, that were added to the insect diet, retarded feeding and inhibited larval growth and were lethal to the larvae at certain concentrations and subsequently caused mortality and shorted the life span of the treated insects and inhibited normal pupal development of *S. frugiperda* larvae. The EO's repellent effect waned with time, indicating that the compounds that cause this effect in the EO are volatile or perhaps are readily oxidized and, therefore, lose their potency. Combinations (mainly Lm + Cs (1 : 1)) of different EOs enhance the activity of the compounds as compared with individual EO in larvicidal repellent bioassay and pupal emergence rate. In conclusion, the EOs from *L. multiflora*, *C. schoenanthus*, and their combinations due to their richness in terpene composites could be used in the future as a biological alternative to synthetic insecticide that cause insect resistance.

Data Availability

The data used to support the findings of this study are included within the article.

Consent

Authors grant all consent to publish the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Dimitri Wendgida WANGRAWA, Antoine WAONGO, and SANOU Drissa conceived the study and prepared the protocols. Dimitri Wendgida WANGRAWA, Drissa SANOU, Antoine WAONGO, and Abdoul Razac SANE analyzed and interpreted the data and drafted the manuscript. Dimitri Wendgida WANGRAWA, Antoine WAONGO, and Drissa SANOU performed data collection and conducted the experiments. Fousséni TRAORE Dov BOROVSKY, Sylvain Nafita OUEDRAOGO, Chloé LAHONDÈRE, and Antoine SANON revised and edited the manuscript to the final form. All the authors have read and approved the final manuscript.

Acknowledgments

We would like to thank McKnight Global Collaboration for Résilient Food Systems of the McKnight Foundation, Minneapolis, MN (grant number 22-119; 2022–2025). We would also like to thank "Laboratoire Centrale d'Entomologie de Kamboinse" at Ouagadougou and "Université Norbert Zongo" for their help during the bioassay. Chloé LAHONDÈRE and Forde Upshur received support from the Fralin Life Sciences Institute and the Department of Biochemistry at Virginia Tech, VA, USA, and we would like to thank Clément Vinauger.

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