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Research Article

Insecticidal and Anti-Feeding Activities of *Cymbopogon schoenanthus, Lippia multiflora,* and *Ocimum americanum* Essential Oils Against Larvae and Pupae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract

The Fall Armyworm (FAW), *Spodoptera frugiperda* is one of the main crop pests in Burkina Faso. The growing resistance of FAW to chemical insecticides and their harmful effects on humans and the environment necessitates the search for biodegradable and eco-friendly substances. Therefore, alternative control methods, such as the use of essential oils (EOs), are important in finding new strategies for the effective management of this pest. This study aims to evaluate the biological properties of EOs extracted from leaves of *C. schoenanthus, L. multiflora,* and *O. americanum* on larvae and pupae of *S. frugiperda*. The activities of these oils were evaluated by topical treatment of different concentrations on L2 larvae and pupae and their effect on food intake. Topical application to L2 caused dose dependent mortality and EO of O. americanum was most effective at 72h with an LC50 of 0.3%. Application of L. multiflora EO caused mortality with a LC50 of 0.4%. EO from *C. schoenanthus* caused LC50 and LC90 of 0.8% and 12.5%, respectively. The oils of L. multiflora and *C. schoenanthus* have been shown to be most effective against pupae. *L. multiflora* and *C. schoenanthus* EOs showed significant effects within 24 h after food intake. The EOs caused inhibition of larval growth and a weight loss of 0.002% per day using 1% *L. multiflora* EOs and 0.003% using 2% C. schoenanthus EOs. The inhibitory effects of these oils could be exploited using integrated pest management for S. frugiperda.

Keywords: Essential oils; *S. frugiperda*; Mortality; Lethal Concentrations; Growth; Anti-Feeding

Introduction

Maize production, like other crops, is experiencing environmental problems enhanced by drought, desertification, floods and edaphic factors [1,2]. In addition to these abiotic phenomena, there are biotic constraints such as crop weeds, diseases and crop pests. The agriculture in Burkina Faso suffers the attacks from crop pests, which limits the production goals that are set by the ministry of agriculture [3]. Crop pests include locusts, grain-eating birds, rodents and the fall armyworm (FAW), *S. frugiperda* (J. E. Smith, 1797). FAW was first observed in Burkina Faso in April 2017 [4]. Since then, agricultural plant damages were mainly caused by the unprecedented spread of the FAW in all regions of the country [3]. It is a polyphagous species with 353 recorded host plants [5]. Females can lay up to 1,500-2,000 eggs during their 2–3-week lifespan [6]. It prefers grasses and, in particular, maize [6-8]. which is present throughout the year in the West African agricultural system. Infestations begin during the developmental stage of maize from the middle to the end of the cycle and cause yield losses ranging from 15% to 73% when 55% to 100% of the plants are infested by *S. frugiperda* [9]. The presence of FAW in Burkina Faso, in connection with its potential for reproduction, propagation and its voracious appetite, constitutes a real problem for the agricultural sector and a threat to the country's food supply [10]. In fact, infestations ramped up from 58,324 ha during the 2017-2018 to 94,108.75 ha in 2019-2020 [10]. According to the same study, during the last crop year 2019-2020, the infestation rate reached an average of 68.60% of the areas surveyed [10]. Faced with this situation, control methods are currently being implemented against this emerging crop pest. It mainly consists of the use of inert substances (ash, sand), cultural, biological and physical controls and synthetic insecticides. Among these control methods, chemical control by synthetic insecticides is the most widely used against *S. frugiperda*. Unfortunately, the use of these insecticides has several drawbacks such as pollution of food chains and the environment, intoxication of producers and consumers and increased pest resistance to synthetic insecticides [11,12].

In view of the drawbacks associated with the use of synthetic insecticides, the search for environmentally friendly control methods becomes urgent. Thus, it is necessary to seek promising control alternatives that minimize the harmful effects of synthetic insecticides [13]. The use of plant extracts can reduce the cost of agricultural production, environmental risks and dependence on synthetic insecticides [12]. Indeed, several substances derived from plants have shown to be effective pest control agents, exerting different biological effects, such as repulsion, food and growth inhibition, changes in the insect pest hormonal system, morphogenetic changes, disturbances in sexual behaviour, sterilization, prolonged immature state, adult mortality and reduced fertility, among others [14-16].

To this end, several essential oils (EOs) with insecticidal properties have been evaluated testing biological, physiological and behavioural parameters of moths of the genus *Spodoptera* demonstrating the potential of these substances as pest control agents [15,17,18]. EOs from *Siparuna guianensis* showed high toxicity *in vitro* to a resistant strain of *S. frugiperda* by necrotic and apoptotic effects [19]. The secondary metabolites of certain EOs such as citral from *Cymbopogon flexuosus* [20], Ar-turmerone from *Curcuma onga* [21] and (+/-) -catechins, a triglyceride, and cedrelone from *Toona ciliate* [22] also exhibited insecticidal properties and repellency on *S. frugiperda*. The present study examines the toxicity of EOs of *Cymbopogon schoenanthus*, *Lippia multiflora*, and *Ocimum americanum* on larvae and pupae of *S. frugiperda*. These plants are already known to be toxic to other insects such as weevils and mosquitoes [23-25]. The general objective of our study is to evaluate the biological effects of the EOs of these plants on *S. frugiperda* to control this agricultural pest and subsequently increase maize production.

Materials and Methods

Study site

We carried out our study at the "Laboratoire Central d'Entomologie Appliquée de Kamboinsé" (LCEAK) of the Center for Environmental, Agricultural and Training Research of Kamboinsé (CREAF-K). The study site is located in the province of Kadiogo, at the northeast exit of the city of Ouagadougou on the Ouaga-Kongoussi axis (latitude 12 ° 28 North, longitude 1 ° 32 West and altitude 296 m). CREAF-K is one of the five (05) research stations of "'Institut de l'Environnement et de Recherches Agricole" (INERA).

Insect rearing

The rearing of *S. frugiperda* was carried out in insectarium at a temperature between 26 to 30 ° C, relative humidity of 60 to 80% and a photoperiod of 12:12 h (light: dark). Larvae were placed in glass jars with a capacity of 1L and then fed with fresh maize leaves from a maize field used for this study. The food was daily renewed until pupation.

For the later instar larvae, fresh cut stems were added to the leaves as a food source. The pupae were then placed in cages until emergence. The newly emerged adults were fed 10% diluted honey and cotton wool soaked in water. Fresh maize leaves obtained 30 to 50 days after seedlings (DAS) were placed in each cage to serve as

a substrate for adults 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 without prior contact with insecticides.

Extraction and dilution of essential oils

The EOs of *C. schoenanthus*, *L multiflora*, and *O. americanum* leaves were used in this study. The oils extraction was carried out by hydrodistillation using a Clevenger type apparatus at the "Institut de Recherche en Sciences Appliquées et Technologies" (IRSAT). The plants were grown in the yard of IRSAT. The taxonomic identification of the plants was carried out at the 'Laboratoire de Biologie et d'Ecologie Végétales' (Université Ouaga I Pr Jospeh KIZERBO. Burkina Faso) by Dr. Amadé OUEDRAOGO, and the OUA voucher specimens are kept at the university's 'infobio'. The EOs were diluted with absolute ethanol as the organic solvent. Five concentrations (0.1%; 0.3%; 0.5%; 1% and 1.5%), were used for topical applications. Control groups were treated with ethanol alone.

Identification of Constituents of Essential Oils

The major and minor chemical constituents of *L. multiflora*, *C. schoenanthus*, and *O. americanum* EOs were identified and quantified using gas chromatography - mass spectrometry (GC-MS). An aliquot of 20 µL of 1/5000 diluted solution was pipetted from each EOs sample and placed into a vial with an insert (VWR, Radnor, PA) to be used with 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. After each sample was prepared, they were loaded into the machine using an autosampler (TriPlus RSH, Themo 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 (total run: 28.5 min.). Chromatographed peaks were integrated manually using the Chromeleon software MS quantitative processing method (Thermo Fisher Scientific) and tentatively identified using the online NIST library. Major peaks found with consistently high abundances across multiple samples for each EO were then recorded for comparison across species.

Toxicity test of essential oils on L2 larvae

The toxicity of the oils on L2 larvae was determined by topical application as described by Giongo., *et al.* [22]. The toxicity was determined by testing five concentrations (0.1%; 0.3%; 0.5%; 1% and 1.5%) of each EOs. The solvent was absolute ethanol which was used to treat control groups. Larvae were placed in 15 cm diameter Petri dishes using a single larva in a 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). Aliquots (2 µl) were applied using a micropipette to the dorsal cephalic part of each larva. Ethanol (2 µl) was used as a control treatment. Mortality was daily determined and the food was renewed daily for 3 days. Larvae that did not respond to the touch of a flexible forceps were considered dead. Larval rearing conditions are the same as described above.

Toxicity test of essential oils on pupae

The toxicity of EO on the chrysalis was evaluated by immersing each chrysalis for 5 seconds in EO. Five concentrations of EO in ethanol were used (0.1%; 0.3%; 0.5%; 1% and 1.5%). Ethanol was used to treat control groups. Five treatments corresponding to the five concentrations of 10 pupae per treatment, (i.e., 50 pupae per EO) were used in this study. Adult emergence was evaluated between day 7 and day 15 after treatment with EO, corresponding to the time of adult's emergence in our breeding facility.

Food intake inhibition study of *S. frugiperda* L3 larvae

Anti-feeding tests of uniform size L3 larvae.

S. frugiperda L3 stage was chosen for these tests because from this stage onwards that the feeding rate increases significantly. Preliminary tests were carried out to determine the concentrations that cause a strong and a weak inhibition of food intake. For each EO, five concentrations were tested (0.25%; 0.5%; 1%; 1.5% and 2%). Pieces of corn husks of known mass 30 to 50 days after seedling were immersed for 5 seconds in EO solution corresponding with each treatment. Control groups leaves were immersed in ethanol. The treated solutions on the corn 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 testing. Observations of larvae were made at 12hours intervals for 24hours and the remaining food in the petri dishes was also weighed at 12hours intervals.

Growth inhibition test

Growth inhibition tests were performed using L3 larvae. Five concentrations of EO (0.25%; 0.5%; 1%; 1.5% and 2%) were used to treat pieces of 7cm x 6cm corn husks collected at 30 to 50 days after seedling. The corn husks were immersed for 5 seconds in the EO solution, and the solutions were allowed to dry at room temperature. Leaves were prepared as described above and fed to *S. frugiperda* L3 larvae. 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 the duration of the test (days). A comparison was subsequently made between GR in the treated and GR in the corresponding controls.

Data analysis

The database was tested for normality and homogeneity of variances; these two conditions were first verified before performing analyses of variance (ANOVA). The data on the EO application on L2 larvae and the amount of food consumed by the L3 larvae was assayed by ANOVA and non-parametric Kruskal-Wallis analysis. Significant p-value ($p < 0.05$) determination followed with pairwise. perm. t. test and dunnTest for two-by-two comparisons of means for ANOVA and the Kruskal-Wallis test, respectively. 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 RV "Aide-Memoire" [26] and FSA [27] 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.

Results

Twenty-three compounds with concentrations higher than 0.1% were identified in *C. schoenanthus* EOs (Table 1). The principal compounds of this oil were piperitone (24.81%), β-elemol (20.64%), β-selinenol (15.57%), α-terpinene (10.23%), epi-γ-eudesmol (6.07%) and D-limonene (5%). In *O. americanum* 25 compounds were identified (Table 1) and the main compounds were eucalyptol (37.29%), camphor (13.58%), caryophyllene (9.05%), trans-α-bergamotene (8.36%), α-farnesene (5.85%), α-pinene (5.41%) and 4-thujene (5.04%). Finally, in *L. multiflora* 26 compounds were identified (Table 1) and the main compounds were carvacrol (21.91%), p-cymene (16.15), caryophyllene (14.93%), thymol acetate (13.97%) and γ-terpinene (9.01%).

R. time	Compounds	*C. schoenanthus* (%)	*O. americanum* (%)	*L. multiflora* (%)
12.284	α-Pinene	-	5.41	0.32
12.611	Camphene	-	1.17	-
13.23	4-thujene	-	5.04	-
13.434	β-Myrcene	-	1.22	2.80
13.607	α-Terpinene	10.23	0.11	1.96
13.652	(+)-4-Carene	3.91	-	-
13.723	α-Phellandrene	0.19	-	2.80
14.053	p-Cymene	0.24	-	16.15
14.111	β-Cymene	-	0.36	-
14.169	D-Limonene	5.00	0.38	0.35
14.216	Eucalyptol	-	37.29	4.34
14.454	β-Ocimene	-	-	0.81
14.696	γ-Terpinene	-	0.41	9.01
14.968	trans-β-Terpineol	-	0.15	0.11
15.509	Linalool	-	0.68	0.15
15.937	cis-2-Menthenol	1.11	-	
16.349	Camphor	-	13.58	0.24
16.767	Phellandren-8-ol	0.24	-	-
16.781	δ-Terpineol	-	0.53	-
16.968	Terpinen-4-ol	-	1.06	0.54
17.155	α-Terpineol	1.71	3.36	0.16
17.42	E-Piperitol	0.42	-	-
18.104	Piperitone	24.81	-	-
18.624	Thymol	-	-	3.02

18.645	Bornyl acetate	-	3.33	
18.658	Carvacrol	-	-	21.91
19.529	Thymol acetate	-	--	13.97
19.627	Ylangene	-	-	0.14
20.039	Cedrene	-	0.41	
20.189	Elemene	3.78	-	0.17
20.627	Caryophyllene	1.87	9.05	14.93
20.842	trans-α-Bergamotene	-	8.36	-
21.029	cis-β-Farnesene	-	0.23	0.86
21.121	Humulene	0.14	0.27	1.24
21.552	Germacrene D	-	-	1.64
21.702	γ-Gurjunene	0.48	-	-
21.723	α-Farnesene	-	5.85	-
21.842	Copaene	0.26	-	-
21.957	δ-Cadinene	0.54	-	-
22.236	β-Elemol	20.64	0.33	-
22.818	Caryophyllene oxide	0.87	0.58	1.56
23.277	epi-γ-Eudesmol	6.07	-	-
23.508	Agarospirol	0.48	-	-
23.699	β-Selinenol	15.57	-	-
23.79	β-Eudesmol	-	-	0.45
Total (%)		98.93	99.18	99.53

Table 1: Volatile organic compound composition of the essential oils.

N.B. Compounds representing less than 0.1% in the same row in all essential oils were not listed. -: Not detected; RT: retention time.

Toxicity of the EO on L2 larvae

C. schoenanthus EO contact mortality against L2 larvae was dose dependent as related to the duration time that the EO was in contact with the larvae (P_{24h} = 0.015; P_{48h} = P_{72h} = 0.001) (Table 2). Concentration of 1.5% caused the highest mortality rate at 24 h in L2 larvae of 60%, 66.67% at 48 h and 66.67% at 72 h. Whereas concentration of 0.3% caused the lowest mortality rate of 13.33% in 24 h 16.67% in 48 h and 23.33% in 72 h. The mortality of L2 larvae increased with the duration of the contact with the EO. All concentrations of *C. schoenanthus* EO caused higher mortality than ethanol control (0% in 24 h; 6.67% in 48 h and 72 h) and was statistically significant ($p < 0.05$).

Treatments	Larval mortality rate (%) ± SE		
	24h	48h	72h
Ethanol	00 ± 00 c	6.67 ± 3.33 c	6.67± 3.33 d
0.1%	16.67 ± 3.33 bc	20 ± 5.77 bc	23.33 ± 6.67 cd
0.3%	13.33 ± 8.82 bc	16.67 ± 6.67 bc	23.33 ± 3.33 cd
0.5%	30 ± 00 b	30 ± 00 bc	43.33 ± 6.67 bc
1%	30 ± 5.77 b	40 ± 10 b	56.67 ± 8.82 ab
1.5%	60 ± 5.77 a	66.67 ± 3.33 a	66.67 ± 3.33 a
ANOVA	χ^2= 14.161. df = 5 P = 0.015	$F_{5;12}$=13.6 P=0.001	$F_{5;12}$=15.6. P=0.001

Table 2: Mortality of L2 larvae of *S. frugiperda* after 72 hours contact with the essential oil of C. *schoenanthus.*

The means ± standard errors with the same lowercase letters in the same column are not significantly different (Anova by permutation and Kruskal-Wallis test, α = 0.05). SE= Standard Errors.

Contact toxicity of *L. multiflora* EO with L2 larvae showed a dose-dependent mortality. Mortalities were significant between treatments depending on the duration of the contact time (P_{24h}= 0.01; P_{48h}= 0.01; P_{72h} = 0.011) (Table 3). The concentration of 1.5% EO caused the highest mortality rate (80%) in L2 larvae in 24 hours and 48 hours, and 83.33% in 72h. On the other hand, concentration of 0.1% EO caused lower rates of 26.67% in 24h, 30% in 48h and 33.33% in 72h. The mortality of L2 larvae also increased with the duration of larval contact time and EO concentration. All the treatments with this EO concentration resulted in a greater lethality as compared with mortality to the L2 larvae control treated by ethanol (0% in 24 hours; 6.67% in 48 hours and 72 hours).

Treatments	Larval mortality rate (%) ±SE		
	24h	48h	72h
Ethanol	00 ± 00 b	6.67 ± 3.33 b	6.67 ± 3.33 c
0.1%	26.67 ± 14.53 b	30 ± 17.32 b	33.33 ± 14.53 bc
0.3%	26.67 ± 6.67 b	30 ± 5.77 b	36.67 ± 8.82 bc
0.5%	40 ± 10 b	43.33 ± 12.02 ab	53.33 ± 12.02 ab
1%	40 ± 10 b	50 ± 11.55 ab	53.33 ± 12.02 ab
1.5%	80 ± 5.77 a	80 ± 5.77 a	83.33 ± 3.33 a
ANOVA	χ^2 = 12.511. df = 5. P = 0.028	$F_{5;12}$= 5.53. P= 0.01	$F_{5;12}$= 6.56; P= 0.011

Table 3: Mortality of L2 larvae of *S. frugiperda* at 72 hours after contact with the essential oil of *L. multiflora.*

The means ± standard errors with the same lowercase letters in the same column are not significantly different (Anova by permutation and Kruskal-Walli's test, α = 0.05). SE = Standard Errors

The L2 larval toxicity after contact with the EO of *O. americanum* showed that there is no significant difference in mortality after treatment with different concentrations of EO at 24 hours (P_{24h} = 0.121). However, the statistical data indicated significant differences in mortality after EO treatments at 48 and 72 hours (P_{48h} = 0.004 and P_{72h} = 0.002) (Table 4). During these two periods, the highest mortality rate of L2 larvae was 73.33% at 48h and 80% at 72h when EO (1.5%) was used, and the lowest mortality was 20% at 48h and 36.67% at 72h when EO (0.1%) was used. The mortality of L2 larvae increased with the duration of the contact and the concentration of the EO tested. All the treatments for this EO caused mortalities greater than that of contact of L2 larvae with ethanol (control) at 48 and 72 hours (0% at 24 hours; 6.67% at 48 hours and 72 hours).

Treatments	Larval mortality rate (%) ± SE		
	24h	**48h**	**72h**
Ethanol	00 ± 00 a	6.67 ± 3.33 c	6.67 ± 3.33 c
0.1%	20 ± 5.77 a	20 ± 5.77 bc	36.67 ± 13.33 bc
0.3%	36.67 ± 8.82 a	46.67 ± 8.82 b	53.33 ± 12.02 ab
0.5%	23.33 ± 8.82 a	30 ± 5.77 bc	40 ± 5.77 bc
1%	36.67 ± 16.67 a	43.33 ± 14.53 b	53.33 ± 8.82 ab
1.5%	16.67 ± 8.82 a	73.33 ± 3.33 a	80 ± 5.77 a
ANOVA	χ^2 = 8.718. df = 5. P = 0.121	$F_{5;12}$ = 8.612. P=0.004	$F_{5;112}$ = 7.19. P=0.002

Table 4: Mortality of L2 larvae of *S. frugiperda* after 72 hours of contact with the essential oil of *O. Americanum*.

The means ± standard errors with the same lowercase letters in the same column are not significantly different (Anova by permutation and Kruskal-Walli's test, α = 0.05). SE = Standard Errors.

The calculated lethal concentrations at 50% and 90% (LC_{50} and LC_{90}) show that the three EOs tested were toxic by contact to L2 larvae of FAW (Table 5). The values of the LC depend on the plant that the EOs were obtained from. Of these EOs *O. americanum* was the most toxic with LC_{50} and LC_{90} of 0.3% and 7.7% respectively. Second most toxic EO was from *L. multiflora* (LC_{50} = 0.4% and LC_{90} = 8.3%) and the least toxic EO was from *C. schoenanthus* (LC_{50} = 0.8% and the LC_{90} = 12.5%). Analysis of the confidence intervals shows that there is a highly significant difference between the LC_{50} of *O. americanum* and that of other EOs (P < 0.001).

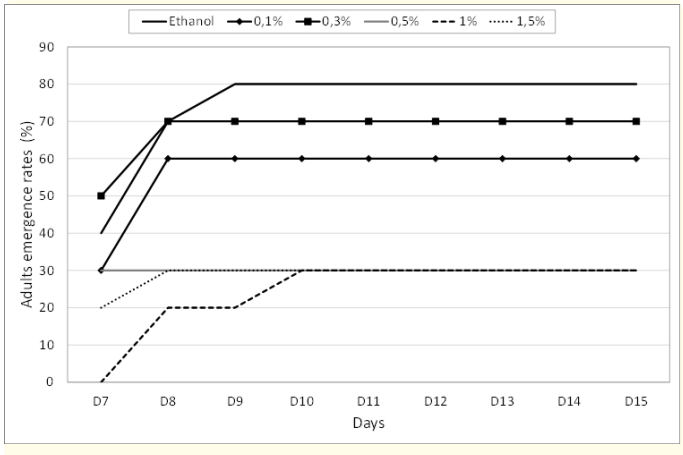
Plants	LC_{50} (%)	LLC-ULC %	LC_{90} (%)	LLC-ULC %	P-value	Chi^2(Wald)
L. multiflora	0.4	(0.2 - 0.7)	8.3	(2.9 - 211.3)	<0.0001	14.09
C. schoenanthus	0.8	(0.5 - 1.4)	12.5	(4.2 - 275.6)	<0.0001	15.87
O. americanum	0.3	(0.1 - 0.5)	7.7	(2.3 - 822.1)	<0.001	10.37

Table 5: Lethal concentrations 50 and 90 (LC50 and LC90), their 95% confidence intervals and the parameters of regression of the larvicidal activity (L2) of essential oils of *L. multiflora, C. schoenanthus O. americanum* on *S. frugiperda*.

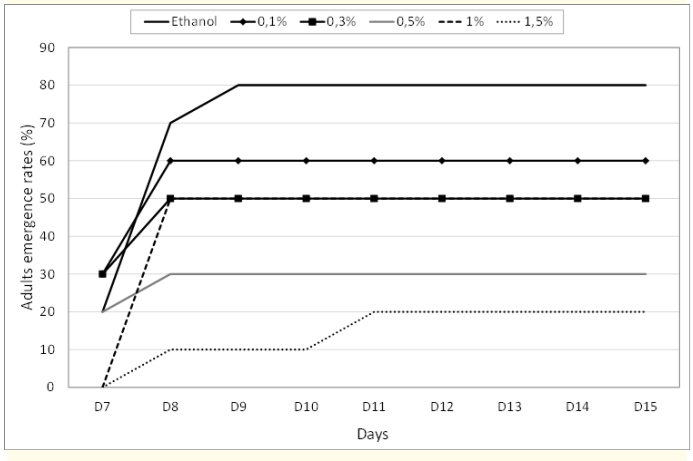
LC: Lethal Concentration; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit

Effect of essential oils on pupae

Figure 1a. shows that adult emergence rates depend on *C. schoenanthus* EO concentrations and are lower when compared with emergence after ethanol treatment. EO concentration (1.5%) caused a maximal emergence of 30% in 10 days as compared with 80% in 9 days that was observed for ethanol (control). Thus, the emergence of *S. frugiperda* is 2.7-fold inhibited by the EO of *C. schoenanthus*. The treatments of pupae with *L. multiflora* EO resulted in low emergence of adults compared with ethanol control (Fig.1b). Indeed, the EO (1.5%) of *L. multiflora* caused a 4-fold decrease in the emergence of adults at 11 days as compared with treatment with alcohol (20% as compared with 80% emergence in 9 days for treatment with ethanol).



a

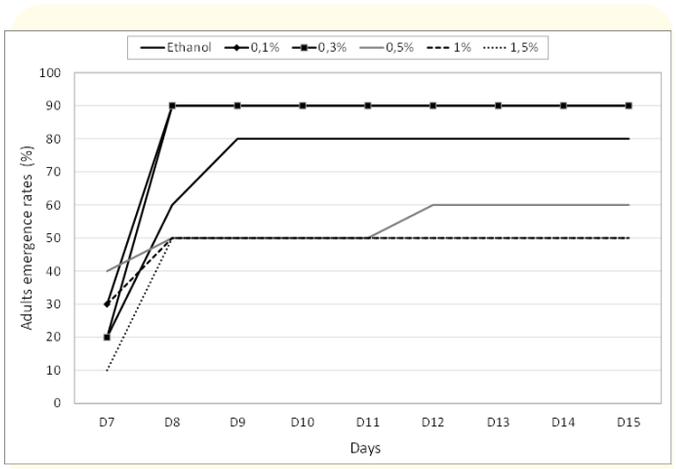


b

Adult emergence rates based on treatment with EO from *O. americanum* are low compared to the control (Figure 1c). Indeed, the emergence rate after treatment with EO (1.5%) is 50% while that of the control is 90% a 1.5-fold decrease when compared with ethanol control. In addition, the emergence after treatment with EO (0.5%) was slow reaching a peak inhibition of 60% in 10 days as compared with 9 days after ethanol (control) treatment.

Effect of EOs on quantity of food intake of L3 larvae of *S. frugiperda*

After 12 hours of treatment with EO, there is no difference between the consumption of control leaves and leaves treated with *L. multiflora* (Table 6). On the other hand, there are significant differences after treatment of corn leaves with EOs (0.25%) from *C. schoenanthus* and *O. americanum* after 12 h. After 24 hours of treatment, the drop in consumption is significant after treatment with 0.5% concentrations of all the EO. The drop in food consump-



c

Figure 1: Adults emergence rates of S. frugiperda after treatment with different concentrations of essential oils on pupae: a. *C. schoenanthus*, b. *Lippia multiflora* and c. *Ocimum americanum*

tion is highly significant (P = 0.0001) after treatment with EO (2%) from *C. schoenanthus* and *L. multiflora,* showing food consumption of 0.057g and 0.056g, respectively at 24h (Table 6).

Rate of food consumption of L3 larvae

All EOs treated L3 larvae showed a reduction in food consumption at 12 hours and at 24 hours after treatment (Table 7). This reduction is 1.4-fold lower after treatment with *L. multiflora* EO (1%) at 24 hours as compared with 12 h. Indeed, a low rate of L3 larvae consumption of 10% of the food substrate treated with *L. multiflora* EO (2%) was observed at 12 h (Table 7). For EOs, there is a highly significant difference between the food consumption after 12h and 24h using EO (1%) treated food substrates.

Effect of EOs on the growth of *S. frugiperda* L3 larvae

Our results show that maize-food treated with different EOs reduce larval growth and was dependent of the EO concentration that the food was treated with (Table 8). EO (1%) reduced

Treatments	Average mass of substrate consumed (g) ±SE					
	12h			24h		
	L. multiflora	*C. schoenanthus*	*O. americanum*	*L. multiflora*	*C. schoenanthus*	*O. americanum*
Ethanol	0.113 ± 0.005 a	0.113 ± 0.005 a	0.113 ± 0.005 a	0.113 ± 0.010 a	0.113 ± 0.010 a	0.113 ± 0.010 a
0.25%	0.112 ± 0.004 a	0.103 ± 0.004 b	0.095 ± 0.004 b	0.0952 ± 0.005 ab	0.098 ± 0.004 ab	0.089 ± 0.005 b
0.5%	0.112 ± 0.010 a	0.086 ± 0.003 c	0.090 ± 0.003 b	0.083 ± 0.009 bc	0.079 ± 0.009 bc	0.093 ± 0.006 b
1%	0.111 ± 0.004 a	0.089 ± 0.003 c	0.083 ± 0.005 b	0.068 ± 0.002 bc	0.070 ± 0.008 bc	0.091 ± 0.005 b
1.5%	0.098 ± 0.003 a	0.084 ± 0.003 c	0.085 ± 0.003 b	0.088 ± 0.003 ab	0.067 ± 0.008 bc	0.077 ± 0.004 b
2%	0.096 ± 0.002 a	0.080 ± 0.002 c	0.081 ± 0.002 b	0.056 ± 0.012 c	0.057 ± 0.010 c	0.081 ± 0.004 b
Probabilities	$F_{5;54}$ = 2.06. P = 0.085	$F_{5;54}$ = 14.387; P = 0.001	$F_{5;54}$ = 9.9486; P = 0.001	χ^2 = 25.18; df = 5. P = 0.0001	$F_{5;54}$ = 6.1247. P = 0.001	$F_{5;54}$ = 4.359. P = 0.002

Table 6: Average weight of corn husks consumed by L3 larvae after 12 hrs and 24 hrs.

The means ± standard errors with the same lowercase letters in the same column are not significantly different (Anova by permutation and Kruskal-Wallis test, α = 0.05). SE= Standard Errors

Treatments		Rate of L3 larvae having consumed the substrate (%)				
	12h			24h		
	L. multiflora	*C. schoenanthus*	*O. americanum*	*L. multiflora*	*C. schoenanthus*	*O. americanum*
Ethanol	90 cd	90 cd	90 c	80 c	80 cd	80 a
0.25%	100 d	100 d	60 b	100 c	100 d	90 a
0.5%	80 cd	80 bcd	60 b	80 c	90 cd	90 a
1%	70 c	70 bc	50 b	40 b	70 c	80 a
1.5%	30 b	60 b	60 b	30 ab	40 b	70 a
2%	10 a	20 a	30 a	20 a	10 a	90 a
Probabilities	χ^2 = 100 df = 5; P < 0.001	χ^2 = 57.143. df = 5; P < 0.001	χ^2 = 32.286; df = 5; P < 0.001	χ^2 = 90.571; df = 5; P < 0.001	χ^2 = 88.462. df = 5; P < 0.001	χ^2 = 4. df = 5. P = 0.549

Table 7: Percentage of L3 larvae that have consumed the substrate.

The means with the same lowercase letters in the same column are not significantly different (Chi-square test of homogeneity, α = 0.05).

significantly larval growth when compared with EOs control ($P < 0.001$). Indeed, all the EOs at high concentration of 2%, reduced larval growth whereas, *L. multiflora* and *C. schoenanthus* strongly reduced the growth of *S. frugiperda* L3 larvae as compared with *O. Americanum* EO (Table 8).

Treatments	Rate of growth (%)		
	L. multiflora	*C. schoenanthus*	*O. americanum*
Ethanol	0.043 ± 0.003 a	0.043 ± 0.003 a	0.043 ± 0.003 a
0.25%	0.039 ± 0.002 ab	0.029 ± 0.007 b	0.044 ± 0.006 a
0.5%	0.032 ± 0.005 b	0.027 ± 0.005 b	0.035 ± 0.006 ab
1%	-0.002 ± 0.0004 c	0.013 ± 0.005 c	0.010 ± 0.003 c
1.5%	-0.003 ± 0.0007 c	0.003 ± 0.004 cd	0.023 ± 0.007 cb
2%	-0.004 ± 0.0005 c	-0.003 ± 0.001 d	0.015 ± 0.006 c
Probabilités	$\chi^2 = 46.35$; df = 5; P < 0.001	$\chi^2 = 37.129$; df = 5. P < 0.001	$\chi^2 = 22.704$; df = 5; P < 0.001

Table 8: Growth rate of L3 larvae of *S. frugiperda* after treatment of substrates with essential oils.

The means ± standard errors with the same lowercase letters in the same column are not significantly different (Kruskal-Wallis test, α = 0.05).

Discussion

The EOs that were tested in our study were toxic against larval and pupal *S. frugiperda*. The level of toxicity depends on the plants that the EOs were obtained from, their concentration, the exposure time and the developmental stage of the tested insect. The lethality of the EOs observed in larvae is probably linked to the action of the chemical compounds present in the tested EOs. Previous studies have shown the lethal effect of EOs against *S. frugiperda* larvae [19,28]. The differences in mortality after treating the insects are probably due to the differences in the chemical compositions of each EO. Indeed, EOs containing monoterpenes, sesquiterpenes and diterpenes exhibiting insecticidal properties was reported by Maggi and Benelli [29]. *O. americanum* EO mainly contains 1,8-cineole, camphor and cis-transpiperitol [30,31]. However, in this study, we found that *O. americanum* contains mainly eucalyptol, caryophyllene, trans-α-Bergamotene and camphor which were reported by previous studies [31,32]. Soro., *et al*. [33]. showed that the EO of *L. multiflora* contains mainly 1,8-cineole, sabinene, and terpinol, but in this study we found that the main components were carvacrol, p-cymene, caryophyllene, thymol acetate and γ-terpinene, showing a different profile. *C. schoenanthus* EO has been reported to contain mainly piperitone, ɖ-2 carene, elemol and limonene [34], which correlates with our results (Table 1). The chemical composition of a given plant can vary and is dependent on many factors including soil acidity, climate (heat, photoperiod, humidity) and the plant part used for extraction [35]. Compounds that are terpenoids, are known for their larvicidal activity [36,37]. Our results corroborate those of Ketoh., *et al*. [34]. who reported that the EO of *C. schoenanthus* and its main constituent piperitone show insecticidal activity against neonate larvae of *Callosobruchus maculatus.* Limonene, made from the oil of *C. schoenanthus*, also found in some citrus fruits, acts as a nerve toxin and contact poison [38]. In addition, limonene is toxic against *Lycoriella ingenua* [39] and D-limonene has insecticidal activity against *Blattella germanica* (L.), *Musca domestica* (L.), *Sitophilus oryzae* (L.) and *Diabrotica virgifera* (Leconte) [40].

The toxicity of *L. multiflora* is probably due to the presence of terpenes, especially caryophyllene, carvacrol, thymol and p-cymene. These components possess insecticidal properties against many kinds of insects including mosquitoes, stored products insects, and crop pests [41]. Traboulsi., *et al*. [42], reported that 1,8-cineole and terpineol are toxic to mosquito larvae (*Culex pipiens* molestus Forskal). Our results are similar to those of Negrini who demonstrated that the EOs of, *Lippia microphylla*, caused 92% mortality after topical application at 15mg/g dose to *S. frugiperda* [15]. This shows the potential for the usage of the genus *Lippia* for pest control, in particular against *S. frugiperda*.

Similarly, Silva., *et al*. [43] showed that the EO of *O. basilicum* is predominantly composed of 95% terpenes such as linalool, 1,8-cineole, and geraniol. Some of these compounds are also found in the EO of *O. americanum* and in our study. These same authors have shown that these compounds cause, by contact, extreme agitation and hypersensitivity to L3 larvae of *S. frugiperda* followed by loss of motor coordination, reduced feeding and death.

A study reported by Ketoh., *et al*. [34] showed that the EO of *C. schoenanthus* is toxic to adults of *C. maculatus,* with a $LC_{50} = 2.7$ µl /L as compared with LC_{50} of 1.6 µl /L for piperitone, its main constituent [34]. This indicates that the biological action of an EO does not depend only on the effect of a single major compound, but on a set of molecules it contains. These molecules can either have synergistic effect by increasing the biological action of the EO, or antagonistic effect that decreases the overall biological effect of the EO components [44,45]. In addition, the actions of EOs are due to terpenes which act as neurotoxins and their biological activities depend on the insect species [46]. Our results show higher LCs of EOs when *L. multiflora* and *O. americanum* were used in contrast to results obtained by Sanon., *et al*. [23], who showed that *L. multiflora* and *O.*

americanum exhibit toxicities towards adult *C. maculatus* with LC_{50} of 0.23 µl/L and 6.44µl/L and LC_{90} 0.71 µl/L and 11.07µl/L. This could be explained by the difference in sensitivity between the two insect species. From the three EOs used in this study, the EOs of *O. americanum* and *L. multiflora* showed the greater toxicity against larval stages in the laboratory.

Our results show that treated *S. frugiperda* pupae exhibited delayed emergence or even total inhibition of adult emergence. Inhibition of adult emergence is probably due to the effects of EO chemical compounds which diffuse through the protective epidermal layer of pupae delaying adult emergence or kill the pupa. Our results corroborate those of Chi., *et al*. [47] who showed that the EO of *Castela tortuosa* caused a significant mortality to the pupae of *S. frugiperda* because of the presence of terpenes [47]. In addition, Shadia., *et al*. [48], found that the association of the different constituents of the *O. americanum* EO reduced the development of *Agrotis ipsilon* pupae. We did not obtain 100% inhibition of adult emergence like Baskar., *et al*. [49], who showed a total inhibition of *S. frugiperda* adult emergence with 5% of hexane extracts from *Couroupita guianensis*. The differences in these results may be due to the high volatility of the EOs than the organic solvent extracts used by Baskar., *et al*. [49] reducing the contact time of the EOs with the pupae.

Our tested EOs reduced the feeding of the L3 larvae and caused mortalities at high concentrations. The EOs of *L. multiflora* and *C. schoenanthus* significantly reduced feeding at concentrations of 1.5% and 2%. This may be due to a strong deterrence that the EOs exhibit that combined repellence and anti-feeding properties against *S. frugiperda* larvae, resulting in low food consumption. Akhtar., *et al*. [44] reported that the species-specific response of an insect as to whether or not to feed depends on how the chemical interaction between all the constituents of a mixture is detected by the sensilla taste. Previous studies have shown the repellence and anti-feeding effect of EOs on the genus *Spodoptera* [50]. Caballero., *et al*. [51], showed that neo-clerodanic diterpenes and sesquiterpenes, also contained in our EOs, and limonoids have an anti-appetite activity against another species of the same genus, *Spodoptera exigua*. Another study on EOs conducted by Villafañe, also showed that the EO of *Citrus aurantium* incorporated in the diet of L2 larvae of *S. frugiperda* discouraged the feeding of 46% of these larvae at a dose of 250µg per gram of diet [52]. The mortality observed in the anti-feeding test could be due to toxicity by fumigation or by ingestion. Toxicity by fumigation in particular constitutes a promising avenue in the fight against *S. frugiperda*. To this end, Negrini reported that the effect of volatiles would be of benefit in controlling FAW, which usually lurks inside the canister of corn [15]. The reduction of L3 growth by EOs could be due also to more complex biochemical processes that deserve further investigation.

The Eos of *L. multiflora* showed greater sublethal activity leading to a daily weight loss of 0.002% per larva with a concentration of 1% against a weight loss of 0.003% per day with *C. schoenanthus* EO (2%) and a weight gain of 0.015% for *O. americanum*. These results are due to the anti-nourishing properties of these oils over a feeding period of 11 days. Our results show the effectiveness of EOs compared to aqueous extracts of *Copaifera langsdorffii* (Fabaceae) mixed with an artificial diet used by Sâmia., *et al*. [11]. These authors indicated that these aqueous extracts lead to weight gain in the larvae depending on the treatments during 17 days of feeding. Molecules from another plant family such as scopoletin isolated from the branches of *Trichilia pallens* and the triglyceride isolated from the branches, fruits and stems of *T. ciliata,* caused a reduction of 23% and 24% respectively in the larval weight of *S. frugiperda* [22].

Conclusion

The results reported by this study show that the EOs tested exhibit biological properties for pest control. These EOs are promising for future control of *S. frugiperda* by cuticle contact of L2 larvae and pupae. The EO of *L. multiflora* and that of *C. schoenanthus* show toxicity within 24 hours and inhibition of food consumption in L3 larvae. The three EOs also cause reduction in larval growth; *L. multiflora* EO is the most effective than the other two EOs that were tested.

These different properties show not only the efficacy but also the diversity of the insecticidal action of the EOs of *L. multiflora*, *C. schoenanthus* and *O. americanum* against the different developmental stages of *S. frugiperda*, an agricultural pest insect. The EOs of these plants are promising alternatives to the synthetic insecticides currently in use, that rapidly cause resistance and tolerance in *S. frugiperda* in Africa and beyond.

Declarations

- **Ethics approval and Consent to participate**: Not applicable.
- **Consent for publication:** Authors grant all consents to publish the manuscript.
- **Data availability and material:** The data used to support the findings of this study are included within the article.
- **Competing interests:** The authors report no conflict of interest.

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- **Author contributions:** Dimitri Wendgida WANGRAWA, Antoine WAONGO and Fousséni TRAORE conceived the study, prepared protocols. Dimitri Wendgida WANGRAWA, and Antoine WAONGO analyzed and interpreted the data and drafted the manuscript. Dimitri Wendgida WANGRAWA, Antoine WAONGO, Forde Upshur and Fousséni TRAORE did data collection and conducted experiments Zakaria ILBOUDO, Athanase BADOLO, Dov BOROVSKY, Chloé LAHONDÈRE and Antoine SANON revised and edited the manuscript to final form. All authors read and approved the final manuscript.

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