 doi:10.20944/preprints201909.0121.v1

GC/MS profiling and insecticidal potential of essential oils from *Thymus vulgaris* (Lamiaceae) and *Cymbopogon citratus* (Poaceae) against tomato borer, *Tuta absoluta*

Marie Danièle T. Ngongang[1], Pierre Eke[1], Modeste Lambert Sameza[2], Champlain Djiéto-Lordon[3] and Fabrice Fekam Boyom[1*]

[1]Antimicrobial & Biocontrol Agents Unit (AmBcAU), Laboratory for Phytobiochemistry and Medicinal Plants Studies, Department of Biochemistry, Faculty of Science, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon

[2] Laboratory of Biochemistry, Department of Biochemistry, Faculty of science, University of Douala, P.O. Box 24157, Douala, Cameroon

[3]Laboratory of Zoology, Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon

*Corresponding author at: Antimicrobial & Biocontrol Agents Unit (AmBcAU), Laboratory for Phytobiochemistry and Medicinal Plants Studies, Department of Biochemistry, University of Yaoundé I, 2nd Bldg, Annex, Faculty of Science, Ngoa Ekelle High School Street, P.O. Box 812, Messa-Yaoundé, Cameroon. Email: fabrice.boyom@fulbrightmail.org (F.F. Boyom)

 doi:10.20944/preprints201909.0121.v1

Abstract

This study aimed to determine the Gas Chrommatography (GC)-Mass Spectrometry (MS) profiles and insecticidal activity of essential oils (EOs) from *Thymus vulgaris* (Thyme) and *Cymbopogon citratus* (Lemongrass) against the invasive and devastating pest, *Tuta absoluta* (*T. absoluta*) through contact and fumigation routes. We found out that thyme oil was predominantly constituted of Thymol (22.16%), α-Pinene (15.35%) and p-Cymene (13.54%) whilst Neral (21.41%), Geranial (21.36%) and β-Myrcene (9.74%) were the major constituents of lemongrass oil. Lemongrass oil exhibited higher insecticidal efficiency irrespective of application mode with 50% lethal dose (LD_{50}) values of 35.8 and 72.2 $\mu L.L^{-1}$air on contact and fumigation routes, respectively. Lemongrass oil also lengthened pupal duration at all tested doses irrespective of application routes. The overall responses of Lemongrass oil surpassed that of the reference insecticide (Lynx®: Lambda-cyhalothrine; Acetamipride). Thus, the recorded data clearly showed the acute and long-term insecticidal effects of the studied EOs, though a greenhouse and open field trials are required prior to the validation of this approach as remediation measure for Integrated Pest Management (IPM) for tomato borer control in Cameroon and elsewhere.

Keywords: Tomato borer, essential oil, GC-MS profile, larvicidal effects.

1. Introduction

Tomato (*Solanum lycopersicum* (Linné)) is a wordwide grown vegetable with a substantial economic value [1]. The nutritional and therapeutic properties of its fruits have raised it beyond all other cultivated legume fruits worldwide. In Cameroon, tomato fruits rank second among produced fruits behind sweet banana [2]. Beside the wide array of diseases which hinder tomato productivity, an invasive pest, known as “South American tomato leaf miner”, “tomato borer” or *Tuta absoluta* [(Meyrick, 1917) (Lepidoptera: Gelechiidae)] has been reported as one of the key devastative pests of solanaceous plants of worldwide significance [3,4,5]. In early 2010’s, this oligophagous insect pest was firstly reported in Bangangté locality, West Region of Cameroon (5°8′47.07″ N, 10°31′26.04″ E) and subsequently rapidly spread in main Cameroonian’s tomato production bassins with substantial yield declines. Till date, despite its outbreak in some commercial fields in the western highlands and southern Plateau’s production hot spots, the pest has not received any attention.

The insect (*T. absoluta)* life cycle comprises four developmental stages: egg, larvae, pupae and moth. Larvae is the only harmfull stage. The former feeds, grows and produces many

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

galleries on soft tissues of the aerial parts of tomato plant such as leaves, stem, flowers and fruits. These damages involve significant qualitative and quantitive yield declines which could culminate at as high as 100% if no efficient control measures is undertaken [6].

Elsewhere, the attention has been paid to the burden of tomato borer [3, 6, 4]. However, very few concerns were raised concerning the frequent spray of synthetic insecticides like Indoxacarb, Coragen and Triflumuron in gardens regardless of the drawbacks inherent to constant and inappropriate use of these agrochemicals on the non-target organisms and the environment as a whole. Recent operational approaches to this concern include the development of more ecological control tools encompassing biological control, planting of insect proof varieties and the utilization plant-derived active metabolites with insecticidal properties [7, 8].

Accordingly, essential oils have hold promise of efficiency, sustainability and compatibility with other control measures in the framework of IPM programs [9]. The repellent [10], fumigant [11], antifeedant [12] and contacticide [13] activities of botanical extracts including essential oils are well documented.

Indeed, essential oils from *Thymus vulgaris* and *Cymbopogon citratus* plants that are otherwise used in Cameroon for culinary purposes have been showed to possess insecticidal activity on a large number of arthropod pests [14, 15, 16]. They could therefore be applied in dwarfting the outbreak of the recently reported Tomato borer in Cameroon (unpublished data). This study aimed thus to undertake GC-MS profiling of thyme and lemongrass EOs and evaluation of their insecticidal activity against *T. absoluta* larvae through two different application routes, contact and fumigation.

2. Results

2.1 Characterization of essential oils

The EOs from thyme and lemongrass were obtained with respective yields of 0.3% and 0.2% (w/w) and had pale-yellow and clear-yellow colorations respectively. The GC/MS profiling of both EOs from *T. vulgaris* and *C. citratus* indicated a total of 53 and 44 compounds respectively, representing 99.7% and 98.6% of total detected components (Table 1). Specifically, thyme oil was predominently constituted of Thymol (22.16%), α-Pinene (15.35%), p-Cymene (13.54%), Linalool (5.52), β-Caryophyllene (4.85%), β-Myrcene (4.85%), Menth-8-en-1-ol (4.02%) and Borneol (4.02%). On the other hand, Neral (21.41%),

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

Geranial (21.36%), β-Myrcene (9.74%), β-Ocimene (8.34%), Linalool (6.62%), Photocitral B (7.18%) and 2-Undecanone (5.15%) were the major components found in lemongrass oil. A total of 19 compounds were shared by both EOs, quantitatively representing 36% and 43% of the Thyme and Lemongrass oils respectively.

Table 1. Chemical composition of *T. vulgaris* and *C. citratus* essential oils.

No	RI	Compounds	Percentage (%)	
			T. vulgaris oil	*C. citratus* oil
1	913	Cyclofenchene	-	0.02
2	915	α –Thujene	3.19	-
3	920	α-Pinene	15.35	0.67
4	929	Camphene	1.73	0.02
5	940	1-Octen-3-ol	3.01	-
6	948	β-Myrcene	4.85	9.74
7	954	1,2-Dimethylcyclohexane	-	0.02
8	958	α-Phellandrene	0.78	-
9	964	α-Terpinene	-	0.19
10	973	Benzene, 1, 2, 4, 5-tetramethyl	3.53	-
11	977	p-Cymene	13.54	0.35
12	979	β-Ocimene	0.15	8.34
13	1005	Linalool	5.52	6.62
14	1008	Menth-8-en-1-ol	4.02	-
15	1011	Photocitral B	-	7.18
16	1016	Piperitol	0.18	0.06
17	1026	Citronellal	-	1.3
18	1027	Photocitral A	-	0.35
19	1028	Camphor	1.07	-
20	1036	Borneol	4.02	-
21	1048	α-Terpineol	1.51	-
22	1050	cis-Carveol	-	0.09
23	1052	Dihydrocarvonone	0.21	-
24	1066	Citral B (neral)	-	21.41
25	1075	Citral A (geranial)	0.42	21.36
26	1082	2-Undecanone	-	5.15
27	1094	3-Methyl-4-isopropylphenol	0.09	-
28	1099	Geraniol	2.09	1.62
29	1100	Thymol	22.16	-
30	1101	Eugenol	0.11	3.45
31	1102	Nerol	0.14	1.05
32	1103	3-Allylguaiacol	-	0.08
33	1105	Lavandulol	0.09	0.82
34	1109	Isobornyl acetate	0.2	3.08
35	1110	Copaene	0.12	-
36	1114	β-Bourbonene	0.35	-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

37	1115	β-Elemene	-	0.03
38	1116	1, 3, 5- Trimethoxybenzene	0.07	-
39	1120	Safranal	-	0.11
40	1121	α-Gurjunene	0.11	-
41	1125	Caryophyllene	4.85	0.14
42	1128	Cis-β-Farnesene	-	0.03
43	1135	Humulene	-	0.06
44	1136	Alloaromadendrene	0.13	-
45	1138	2-Tridecanone	-	0.41
46	1140	γ-Murolene	0.37	-
47	1142	Nerolidol	-	0.02
48	1144	Isoledene	-	0.1
49	1145	Germacrene	2.27	0.03
50	1147	Eugenyl acetate	-	3.13
51	1150	δ-Cadinene	0.03	0.08
52	1152	Cubenene	0.95	
53	1154	Farnesol	0.21	0.07
54	1156	α-Calacorene	0.03	-
55	1160	n-Propyl cinnamate	-	0.01
56	1163	Cetane	0.03	-
57	1167	Aromadrene	0.12	-
58	1170	Caryophyllene oxide	0.53	0.15
59	1172	trans-1,2-Cyclohexylene sulfite	-	0.02
60	1175	Isobutyl cinnamate	-	0.04
61	1178	Calarene	-	0.83
62	1179	8-epi-γ-Eudesmol	0.17	-
63	1182	Cadinol		0.04
64	1185	α-Cadinol	0.5	0.13
65	1187	Longifolenaldehyde	0.06	-
66	1188	6-epi-Shyobunol	0.06	-
67	1189	α-Elinene	-	0.07
68	1190	Farnesol (e), methyl ether	0.1	-
69	1193	1-Methylbicyclo [3.2.1]-octane	0.01	-
70	1204	Eicosane	0.5	-
71	1205	Isoamyl cinnamate	-	0.12
72	1223	6-epi-Shyobunol	0.03	-
73	1225	Methylprednisolone Acetate	0.02	-
74	1256	Caproic anhydride	0.02	-
75	1260	Geranylgeranyl diphosphate	0.04	-
76	1268	Isodurenol	0.04	-
77	1275	Citronellyl phenylacetate	0.01	-
78	1282	2-(1-adamantyl)-4-Bromoanisole	0.01	-
Total	-	-	99.7	98.6

RI: Retention index; components were identified based on RI and GC-MS data and listed according to their order of elution on solid phase. % = Percent peak area of essential oil constituents.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

2.2 Insecticidal effect of essential oils against T. absoluta

2.2.1. Contact toxicity assay

Larvicidal effect of the essential oils on *T. absoluta*: EOs displayed significant ($p<0.001$) larvicidal effect through direct application on fourth instar larvae. The mortality rate was a function of concentration ($r = 0.71$ and 0.72 respectively for thyme and lemongrass EOs) (Figure 1). Indeed at 336 $\mu L.L^{-1}$air, the lemongrass or thyme oils totally cleared the larvae (mortality rate =100%). Moreover, the LD_{50} and LD_{90} values were respectively 35.8 $\mu L.L^{-1}$air and 304.8 $\mu L.L^{-1}$air for lemongrass oil and 63.0 $\mu L.L^{-1}$air and 285.6$\mu L.L^{-1}$air for thyme oil. (Table 2). The Duncan's paired-based comparison test showed that lemongrass oil performed better than thyme oil as it showed lower LD_{50} (35.8 $\mu L.L^{-1}$air) compared to thyme oil (63.0 $\mu L.L^{-1}$air).

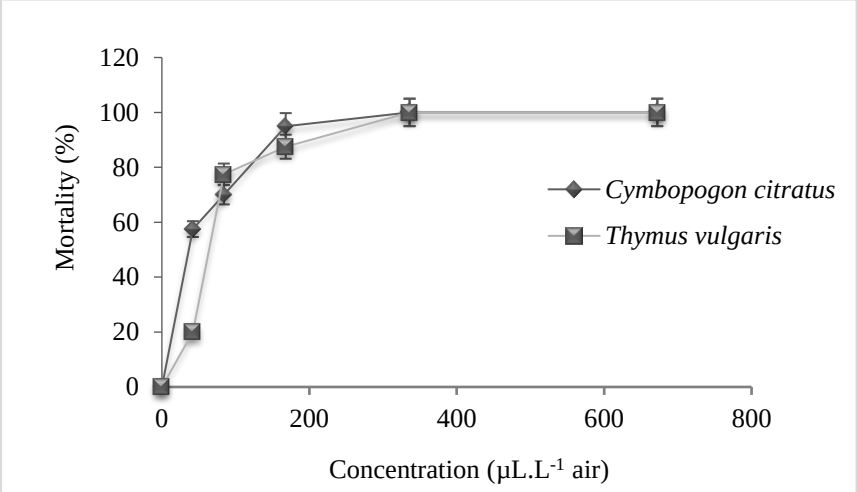

Figure 1. Percentage of mortality (± SD) of *T. absoluta* larvae exposed to increasing concentrations of thyme and lemongrass oils through contact toxicity. Vertical bars represent standard deviation means.

Table 2. LD_{50} and LD_{90} values of thyme and lemongrass oils on fourth larval instar of *T. absoluta* after 4h exposure by contact toxicity.

Essential oils	Inhibition parameter (Range) ($\mu L.L^{-1}$ air)	
	LD_{50}	LD_{90}
Thyme oil	63.00 (62.72-63.28)	285.60 (284.75-286.45)
Lemongrass oil	35.80 (34.39-37.21)	304.80 (303.95-305.65)
Mean difference	27.2	-19.2
p values	0.001**	0.004*

* and ** denote significant difference at $p \leq 0.01$ and $p \leq 0.001$ respectively, given by the Duncan's test.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

Larval knockdown speed when exposed to sub-lethal concentrations of essential oils: The knockdown kinetic of the larvae at sub-lethal doses of EOs was evaluated (Figure 2). The results showed that for each dose, the knockdown occurred within 0 to one-hour time interval after exposure. Beyond one hour, no significant difference was observed with exposure time. Nevertheless, regardless to EOs, the knockdown rate at 42 $\mu L.L^{-1}$air was significantly lower compared to 84 and 168 $\mu L.L^{-1}$air ($p < 0.001$). But there was no significant difference between doses 84 and 168 $\mu L.L^{-1}$air ($p > 0.001$). The KT_{50} and KT_{90} of lemongrass and thyme oils were respectively 0.68 and 0.66h and 0.95 and >4h (Table 3). While no difference was recorded between the KT_{50} of both oils, a statistical difference was however found between the KT_{90} values, indicating a faster action of lemongrass oil compared to thyme oil.

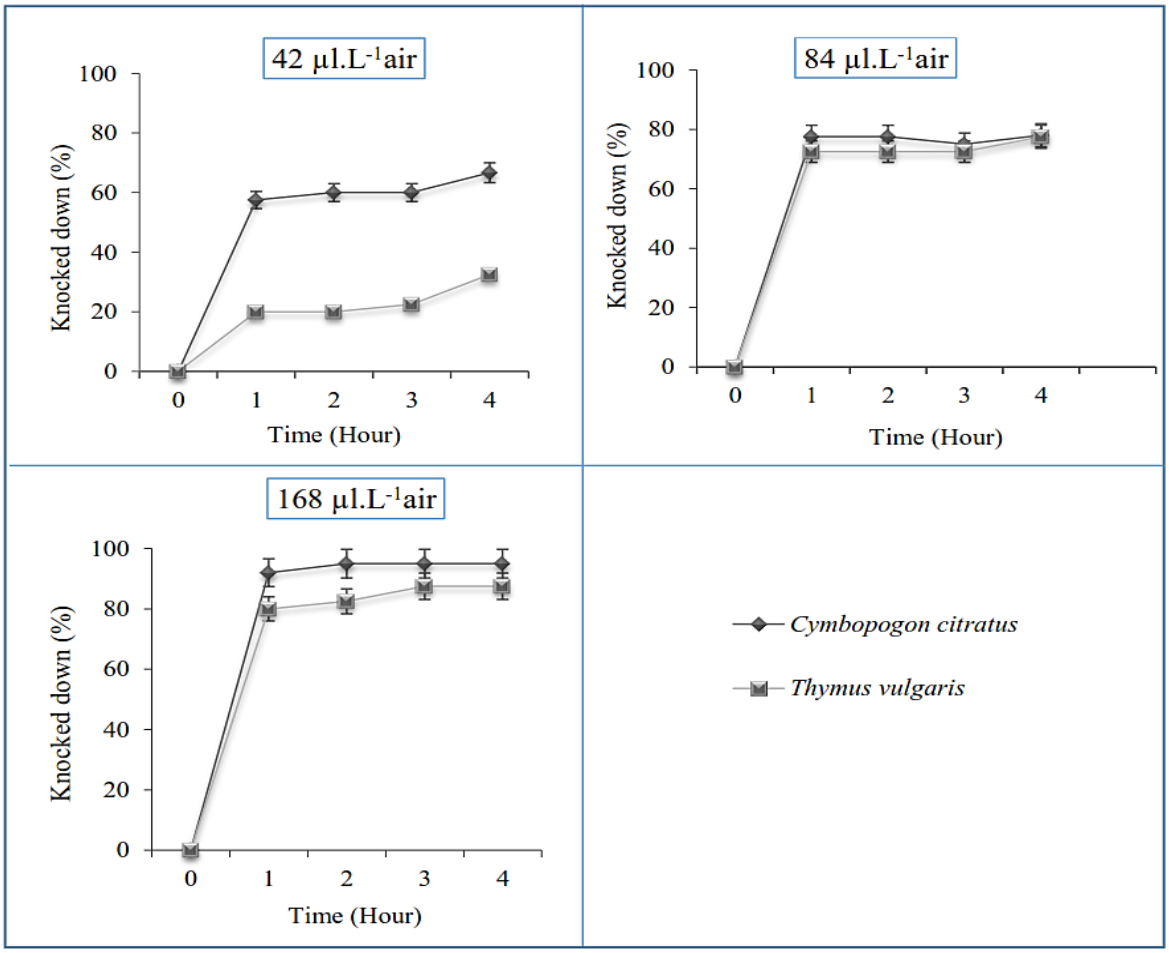

Figure 2. Percentage of larval knockdown by contact toxicity at varying time points when exposed to sub-lethal concentrations of lemongrass and thyme oils. Vertical bars represent standard deviation means.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

Table 3. KT_{50} and KT_{90} values of thyme and lemongrass oils on fourth larval instar of *T. absoluta* by contact toxicity.

Essential oils	Inhibition parameter (Range) (µL.L^{-1}air)	
	KT_{50}	KT_{90}
Thyme oil	0.66 (0.62-0.70)	> 4
Lemongrass oil	0,68 (0.47-0.90)	0.95 (0.94-1.01)
Mean difference	-0.02	Nd
p values	0.905	0.0003*

*Denotes significant difference at $p \leq 0.001$given by the Duncan's test; Nd= Not determined.

Effects of sub-lethal doses of essential oils on larval life cycle: At 42 µL.L^{-1}air, thyme oil significantly increased (3 days) the larval instar duration compared to lemongrass oil (2 days 12 hours) and the negative control (2 days 15 hours), but was statistically equal to the reference insecticide: Lynx® (2 days 17 hours) (Figure 3). For pupal duration, as referred to reference insecticide, both EOs lenghtened the shift from pupae to moth. T. vulgaris oil enhanced the pupal duration (7 days 5 hours) more than the other treatments including the reference insecticide (Lynx®: 6 days 11 hours).

At 84 µL.L^{-1}air, *C. citratus* oil and the positive control did not affect larval duration compared to the negative control. Inversely, *T. vulgaris* oil significantly ($p < 0.05$) lenghtened larval period even more than the reference insecticide (3 days 16 hours). Meanwhile, no difference was observed between pupal duration of *T. vulgaris* and control treatments (Tween 80 and Lynx®), indicating no long-term effects of the former at 84 µL.L^{-1}air. Whereas, exposure to lemongrass oil lenghtened pupal duration (8 days) with reference to the other treatments ($p < 0.001$).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

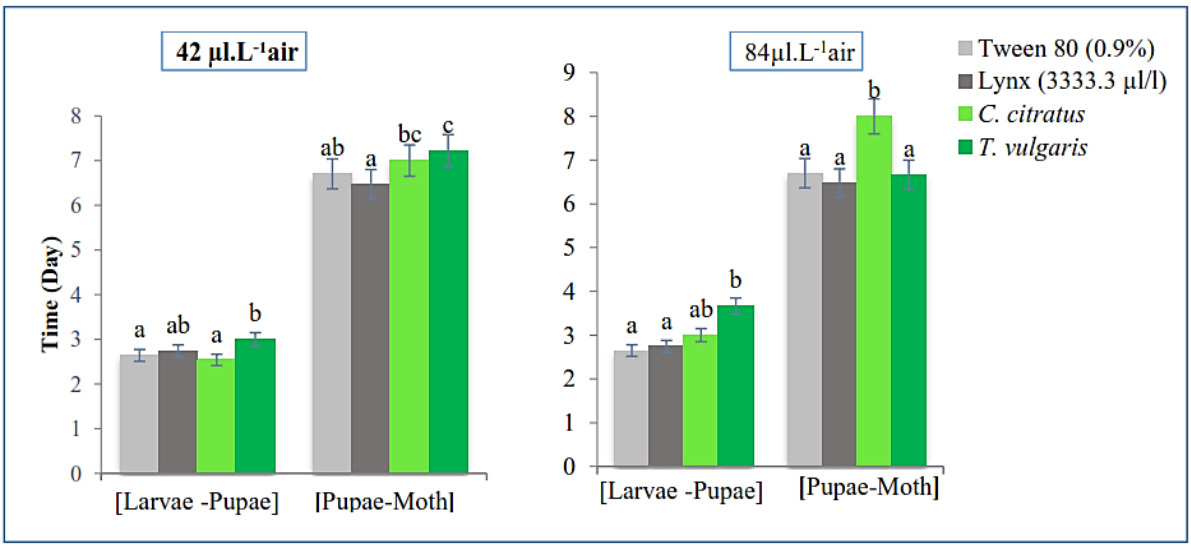

Figure 3. *T. absoluta* larval and pupal durations when exposed to essential oils by contact toxicity. Bars bearing different letters are significantly different referring to the Duncan's based multiple comparison test.

2.2.2 Fumigant toxicity assay.

Effects of the essential oils on *T. absoluta* larval mortality: As with contact toxicity, both oils incited *T. absoluta* larvae dead, in a dose dependent manner (r=0.73 and 0.87 respectively for thyme and lemongrass oils). Globally, lemongrass oil was likely most efficient as it caused 100% larval mortality at 336 µL.L^{-1}air whilst thyme oil needed 672 µL.L^{-1}air to exhibit the same output (Figure 4). In addition, the LD_{50} and LD_{90} were 72.2 and 156.8 µL.L^{-1}air for lemongrass oil and 140.4 and 336.8 µL.L^{-1}air for thyme oil (Table 4). A comparative analysis of the oils based on their respective LD_{50} and LD_{90} clearly indicates a statistically superior larvicidal effect of lemongrass oil over thyme as with contact toxicity.

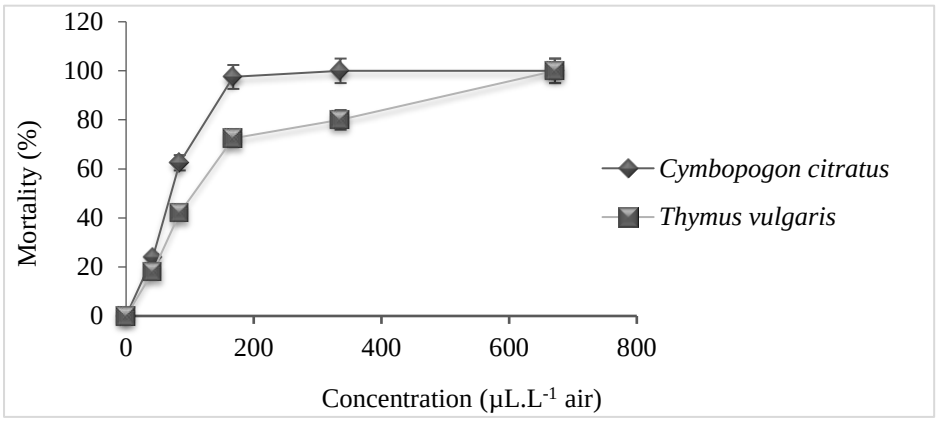

Figure 4. Percentage of mortality of *T. absoluta* larvae when exposed to increasing concentrations of thyme and lemongrass oils through fumigant toxicity. Vertical bars represent standard deviation means.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

Table 4. LD_{50} and LD_{90} values of fourth larval instar of *T. absoluta* after 4h of exposure to *C. citratus* and *T. vulgaris* oils by fumigant toxicity.

Essential oils	Inhibition parameter (Range) ($\mu L.L^{-1}$ air)	
	LD_{50}	LD_{90}
Tyme oil	140.40 (137.57-143.23)	336.80 (336.23-337.37)
Lemongrass oil	72.20 (67.96-76.44)	156.80 (151.14-162.46)
Mean difference	68.2	180
p values	0.003*	0.001**

* and ** denote significant differences at $p \leq 0.01$ and $p \leq 0.001$ given by the Duncan's multiple range test.

Time-dependent larvae knockdown when exposed to sub-lethal concentrations of essential oils: The results revealed that for each sub-lethal dose, the larvae knockdown occurred within 0 to 2 hours contrarily to contact toxicity which occured one hour post treatment (Figure 5). Three and four hours post treatment, no knockdown difference was observed with respect to time. Globally, the mean knockdown percentages at 42, 84 and 168 $\mu L.L^{-1}$air of lemongrass oil were significantly different ($p < 0.001$). The time required to knock 50 (KT_{50}) and 90% (KT_{90}) of larvae were 0.69 and 2.4h for *C. citratus* oil and 0.96 and >4h for *T. vulgaris* oil respectively (Table 5). The Duncan's paired comparaison unveiled that thyme oil needed significantly more time to suppress the larval growth compared to lemongrass oil whose action was somehow quick.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

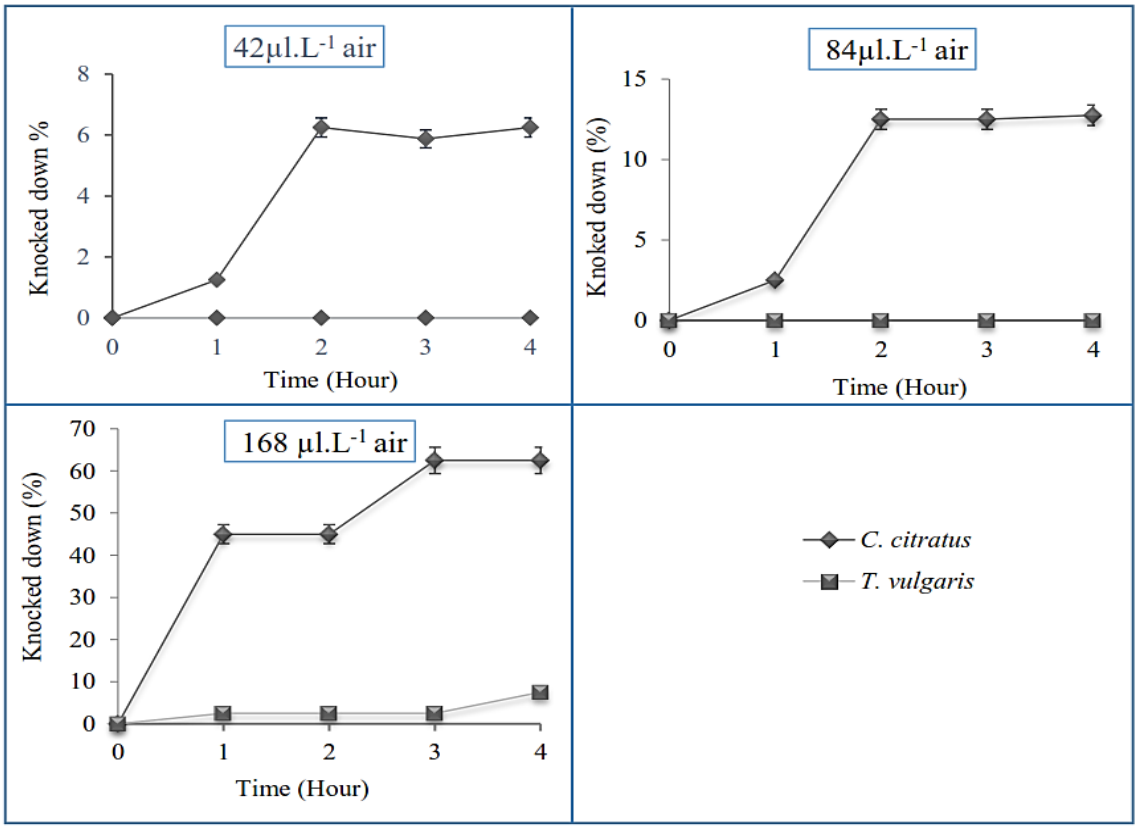

Figure 5. Percentage of larvae knockdown at varying time points upon exposure to differents concentrations of *C. citratus* and *T. vulgaris* oils by fumigant toxicity. Vertical bars represent standard deviation means.

Table 5. KT_{50} and KT_{90} values of Thyme and Lemongrass oils on fourth larval instar of *T. absoluta* by fumigant toxicity.

Essential oils	Inhibition parameter (Range) (µL.L^{-1}air)	
	KT_{50}	KT_{90}
Thyme oil	0.96 (0.89-1.03)	> 4
Lemongrass oil	0.69 (0.66-0.72)	2.4 (2.29 - 2.53)
Mean difference	0.27	Nd
p values	0.0344*	Nd

* denotes signicant difference at $p \leq 0.05$ given by the Duncan's multiple range test.

Effects of sub-lethal concentrations of essential oils on larval development: The larval and pupal duration of survived larvae when exposed to sub-lethal concentrations of EOs was determined (Figure 6). At 42 µL.L^{-1}air, all the tested EOs significantly increased the larval and pupal durations as compared to untreated controls (Tween 80). When treated with thyme and lemongrass oils, their respective effects significantly enhanced the duration of larvae (1

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

day 5 hours; 1 day 6 hours) and pupae (3 days 20 hours; 4 days 11 hours) as compared to both positive (Lynx®) and negative (Tween 80) controls.

At 84 µL.L^{-1}air, although no change was recorded on larval instar duration upon exposition to thyme oil when compared to controls, lemongrass oil significantly augmented the larval duration ($p < 0.001$) relatively to unstressed control. Besides, lemongrasss oil (2 days13 hours) showed higher efficiency compared to thyme oil (2 days 10 hours).

At 168 µL.L^{-1}air, all the tested EOs significantly ($p < 0.001$) enhanced both larval and pupal durations compared to controls (positive and negative controls). Lemongrass oil exerted highly potent effect on pupal duration relative to thyme oil and the reference insecticide (Lynx®).

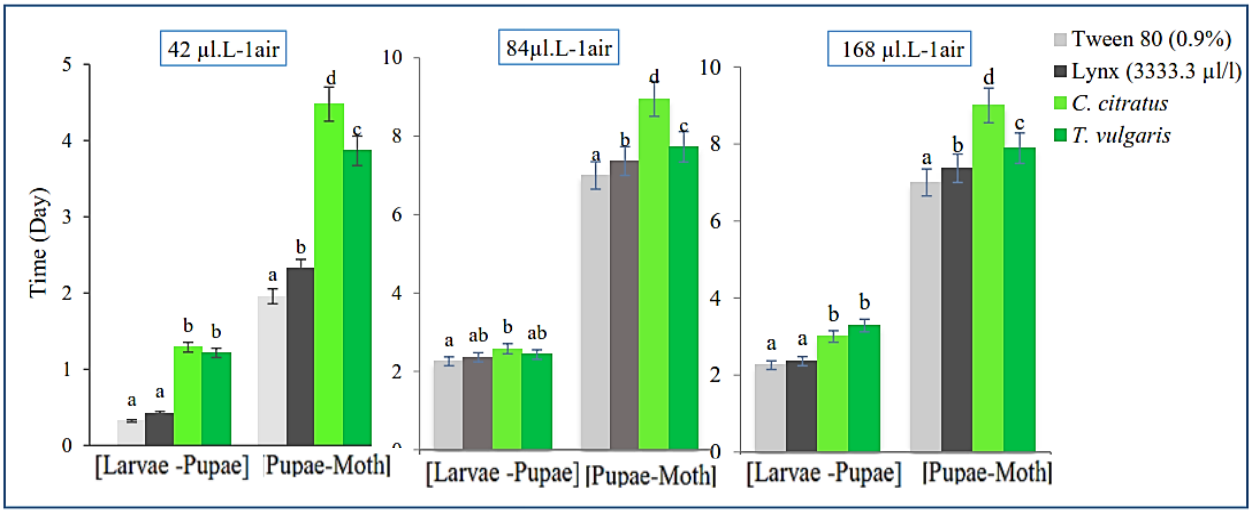

Figure 6. Larval and pupal durations of *T. absoluta* when exposed to essential oils by fumigant toxicity. Mean values (Bars) bearing different letters are significantly different referring to the Duncan's based multiple comparison test.

3. Discussion

The extraction yields of thyme and lemongrass oils were 0.30% and 0.20% respectively. These data are in agreement with the report of Simo et al. [17] (0.32%) for T. *vulgaris* collected in West Region of Cameroon. Other studies by Nguefack et al. [18] reported higher yields (0.65% and 0.70% respectively) from *T. vulgaris* and *C. citratus* dry materials harvested in the West and Centre regions of Cameroon. The analysis of the chemical compositions of EOs revealed the main components in thyme oil to be thymol (22.16%), α-pinene (15.35%) and p-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

cymene (13.54%). On the other hand, neral (21.41%), geranial (21.36%) and β-myrcene (9.74%) were found to be the most abundant in lemongrass oil. Overall, 53 and 44 compounds were identified in *T. vulgaris* and *C. citratus* oils, representing quantitatively 99.70% and 98.59% of their total components respectively. Previous studies by Nguefack et al. [18] have identified thymol (27.2%) as the major component of thyme oil while Tchinda et al. [19] reported geranial (32.2 %), myrcene (27.0 %) and neral (25.7 %) as the principal constituents of lemongrass oil. Such discrepancies in relative compositional abundance of EOs components has been tentatively ascribed to harvest season which can significantly impact on plant metabolism thus essential oils biosynthesis [20]. Through fumigation and direct contact assays, the EOs showed inhibitory action on fourth instar larvae of *T. absoluta*. Lemongrass oil exerted the most potent effect than thyme oil. The observed biological effects could be directly linked to the multitude of components of the EOs and their mechanistic interactions. Previous studies by Tak et al. [21] reported the insecticidal activity of lemongrass oil by contact and fumigant toxicity on the cabbage looper (*Trichoplusia ni*). In addition, Sammour et al. [16] have shown the larvicidal activity of formulated Thyme oil on *T. absoluta*. thus, observed biological activities were presumably thought to be due to the acidic nature of phenolic compounds like thymol, and aldehydes like neral and its isomer (geranial) that enhance their reactivity with most enzyme active sites, leading to their inactivation [22]. This statement was further emphasised by Tak and Isman [8] who found that citral incites reduced frass production, feeding deterrence and larvae growth. At 42 $\mu L.L^{-1}$air, Lemongrass oil delayed larval period through fumigation route meanwhile by contact toxicity, Lemongrass oil did not prolonged larval time. These data further strengthen the inference of application route on the efficacy of extracts on pests. Both oils when tested through fumigation assay lenghtened pupal duration corroborating the findings of El-Mesallamy et al. [23] who reported that basil oil prolonged larval duration of spiny bollworm.

The tomato leaves, stems and fruits mining mediated by *T. absoluta* is a serious constraint to tomato production worldwide. The damage potential, coupled with the rapid multiplication and spread of the insect further justify its high aggressiveness which has incited farmers to augment the insecticidal application rate across the production period to secure acceptable yields [24]. Regardless the fact that insecticides use has helped in curbing food security issues up to some extent, it's being perceived in modern agriculture as limiting factor owing to their negative actions on various components of the environment targeting for instance natural enemies (biological control insects) and pollinisators leading to substantial yield drawbacks.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

Agricultural scientists have therefore adopted the Integrated Pest Management (IPM) approach which could assure nearly same protective effects while preserving human and environmental health. In this sense, plant-derived compounds have been proven to be an integral part of the IPM package as it mimics the mode of action of plant's innate immune system against pests [25]. Despite the aforementioned facts, from the huge number of EOs tested for their insecticidal properties, just few investigations have been directed against *T. absoluta* [25]. More, the available reports tend to report the short term (Acute) toxic effects of EOs whilst the monitoring of the long-lasting effects of these compounds mixtures would be of paramount importance for a confident integration of this technology into the IPM system. This study reports for the first time the insecticidal potential of Lemongrass EO against *T.absoluta.*

4. Materials and methods

4.1. Plants collection and essential oils extraction

Fresh aerial parts were collected from insecticide-free cultivated thyme (*T. vulgaris*) and lemongrass (*C. citratus*) respectively at 8 am in the locality of Mbouda (West Region) on the Mount bamboutos hillside (5°40′11.99″ N, 10°3′2.16″ E) in August 2017 and in the locality of Oyom-abang located in the subdivision of Yaoundé 7 (3°52′22.22″ N, 11°28′48.86″ E) in September 2017. The plants were identified at the National Herbarium of Cameroon where voucher specimens are deposited under the respective reference numbers 42851/CAM and 48536/SRF/CAM for *T. vulgaris* and *C. citratus*.

Freshly harvested samples were subjected to hydrodistillation using a clevenger-type apparatus for approximately 6h. The resulting oils were collected by decantation and dried through anhydrous sodium sulfate column and stored in dark glass bottle until usage. The yield of extraction was determined as follows:

$$\text{Yield (\%w/w)} = [(\text{Mass of essential oil (g)} / \text{Mass of vegetal material (g)}) \times 100].$$

4.2. Determination of the chemical composition of essential oils

The chemical composition of EOs was analysed using Gas Chromatography coupled to Mass Spectrometry (GC-MS) on an Agilent Technologies (7890B GC System and 5977A MSD, Germany) apparatus equipped with a Zebron-5MS column (ZB-5MS 30 m x 0.25 mm x 0.25 um) (5%-phenylmethylpolysiloxane). The analytical conditions were: helium was used as carried gas with a flow rate of 2 ml/min and samples injected at 1ml by splitless mode. The

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

injector and oven temperatures were set at 280°C and 70°C respectively. The temperature of the oven was initially programmed from 70°C to 120°C with a gradient of 15°C/min; from 120°C to 180°C with a gradient of 10°C/min and finally rised to 270°C at the gradient of 20°C/min then maintained for 3 min. Thus, separated compounds flew in MS column at a transfer temperature of 270°C. Mass spectra were obtained from the full scan of the positive ions resulting from ionization of the compounds from the GC with a scanning in an appropriate mass/charge (m/s) range, and operated with an electron impact mode of 200 eV. The components were later on identified by comparing the obtained spectra with standard mass spectra obtained in the same experimental conditions and also to mass spectra of known compounds using Wiley and Nist08 library database available with Xcalibur software.

4.3. T. absoluta and tomato plants rearing

The animal model (*T. absoluta*) used in this study was reared as described in the modified protocol of Benchouikh et al. [26]. Briefly, tomato leaves and stems bearing *T. absoluta* larvae were collected in commercial tomato farms in Foumbot (West Region of Cameroon). Larvae were thereafter transferred individually into Petri dishes afforded with fresh tomato leaves from experimental pesticide-free garden, and followed up on daily basis until moth's emergence. The moths were then realeased into an egg-laying cage (80×160 × 60 cm) along with young trap tomato plants grown in pots for oviposition. The rearing conditions were: 25 ± 2°C, 65 ± 5% RH, and photoperiod of 16/8 hours light to dark cycles. The adults were provided with energy source by soaking cotton wool in 10% honey solution and placing it in rearing cages. After hatching of eggs, the first instar larvae were collected individually into petri dish on fresh tomato leaves and maintained in rearing conditions until reached the fourth instar larval stage which was used for bioassays.

The tomato (cv. Rio Grande) plants utilized for *T. absoluta* rearing, as well as for the bioassays, were grown from seeds in pot culture in screenhouse and at the experimental field of the Antimicrobial and Biocontrol Agents Unit, University of Yaoundé I, Cameroon.

4.4. Preparation of test solutions of essential oils and reference insecticides

Stock solutions of thyme and lemongrass essential oils were prepared by dissolving pure oils in Tween 80 (Sigma Aldrich) (1:9 v/v). Then, two-fold dilutions were made so as to obtain test concentrations raging from 42 to 672 µL.L^{-1}air. The reference insecticide: Lynx®

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

(Lambda-cyhalothrine at 15g/l and Acetamipride at 20g/l) was prepared according to the manufacturer's inctructions (Sun valley Hall Limited, Hong kong) by dissolving the liquid form in sterile distilled water so as to get a final concentration of 3333, 33µL/L which is the recommended dose for farm application against *T. absoluta*.

4.5. Assessment of the insecticidal effects of the essential oils.

Contact toxicity assay of the essential oils: The contact toxicity of the evaluated essential oils on *T. absoluta* larvae was assessed as described by Slimane et al. [13]. In fact, 10 fourth intar larvae per replicate were placed on fresh tomato leaflets into 9 cm Petri dishes, then, EOs were drop-inoculated onto the larvae so as to get final test concentrations from 42 $\mu L.L^{-1}$air to 336 $\mu L.L^{-1}$air. Petri plates were immediately sealed with parafilm tape and maintained in rearing conditions as described above. The control plates were provided with 0.9% Tween 80 for negative control and 3333, 33µL/L Lynx® for positive control. Four replications were prepared per concentration and the overall experiment was repeated twice. The plates were subsequently examined each hour and the number of larvae knocked down (paralysed larvae) was recorded and expressed in terms of percentage over the initial larvae number per treatment [27]. Also, the time required to knock down 50% and 90% (KT_{50} and KT_{90}) of larvae was determined. Thereafter, the larvae knocked down were transfered onto fresh tomato leaves and incubated for 24 more hours under same rearing conditions. The larvae which could not react to the pressure of the brush upon the incubation period on fresh tomato leaves were considered as dead [4]. The mortality rate was then calculated as described by Molan et al. [28]:

$$\text{Mortality (\%)} = 100 \text{ (Number of dead larvae / total number larvae)}$$

In addittion, the living larva were inspected daily to assess the effect of essential oils on pupation. Doing so, living larva were transferred individually into dishes and incubated till moth's emergence. Then, larval and pupal duration were recorded [23].

Fumigant toxicity assay of the essential oils: The fumigant toxicity of the tested EOs on *T. absoluta* larvae was assessed following the protocol described by [4]. Accordingly, ten larva per replicate were transferred on fresh tomato leaves laying into a Petri dish. Whatman No. 1 filter paper disk was fixed onto the inner surface of plates lids and impregnated with different volumes of essential oils so as to get final concentrations range from 42 $\mu L.L^{-1}$air to 672 $\mu L.L^{-}$

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 September 2019 doi:10.20944/preprints201909.0121.v1

[1] air. Negative control plate was provided with 0.9 % Tween 80 and positive control with 3333, 33µL/L Lambda-cyhalothrine, Acetamipride (Lynx®). Four replications were prepared per treatment, and the overall experiment was repeated twice over time. The number of larvae knocked down as well as the KT_{50} and KT_{90} were determined; the mortality rate was recorded as described above and the larval and pupal durations were determined at sub-lethal doses as well [23].

4.6. Statistical analyses

The results were expressed as means ± standard deviation (SD). The one factor ANOVA coupled to the Duncan's Post HOC test was used to measure the distance among means values. The strength of the relationship between concentrations and the observed responses was tested with the Pearson correlation. The LD_{50} and LD_{90} as well as KT_{50} and KT_{90} values were graphically determined using the Statgraphics Plus 5.1 statistical package.

5. Conclusion

This study unveils the larvicidal potential of two essential oils from Cameroonian thyme and lemongrass that were tested through direct contact and fumigation routes on fourth instar larvae of *T. absoluta*. Results indicated that lemongrass oil has stronger larvicidal effect irrespective of application mode. Likewise, regardless of the applicaton mode, sub-lethal doses of both EOs altered significantly the insect pest biolology by lengthening its life cycle. This potency of thyme and lemongrass oils indicate their potential to impair the development speed of *T. absoluta* and thus the reduction of their population density in the farm with the related destructive action on tomato plants. These attributes offer more hopes towards an effective and sustainable control strategy against *T. absoluta* be it singly in greenhouse-based agriculture or combined with available control measures for a better output in open field trials.

Acknowledgments:

This study was partly supported by the Seedling Lab's Instrumental Access Grant (SL2012-2) under Professor FEKAM BOYOM Fabrice. This work also received materials and equipment support from the Yaoundé- Bielefeld Bilateral Graduate School for Natural Products with Antiparasite and Antibacterial Activity (YaBiNaPA).

The authors thankfully acknowledge Mr. NANA Victor for plant specimen idenfication.

Author Contributions: Conceptualization, Champlain Djiéto-Lordon and Fabrice Fekam Boyom; Methodology, Marie Danièle T. Ngongang; Validation, Champlain Djiéto- Lordon; Formal Analysis, Pierre Eke; Resources, Champlain Djiéto- Lordon, Fabrice Fekam Boyom and Marie Danièle T. Ngongang; Data Curation, Champlain Djiéto-Lordon and Pierre Eke. Original draft Preparation, Marie Danièle T. Ngongang; Writing – Review & Editing, Pierre Eke, Modeste Lambert Sameza, Champlain Djiéto- Lordon, Fabrice Fekam Boyom; Supervision, Fabrice Fekam Boyom.

Conflicts of Interest: The authors declare no conflicts of interest.

References :

1. Wakil, W.; Brust, G.; Perring, T. Tomato and Management of Associated Arthropod Pests: Past, Present, and Future. In *Sustainable Management of Arthropod Pests of Tomato*, 1st ed.; Wakil, W., Brust, G., Perring, T.; Academic Press: United States, United States, 2017; pp. 3-10.

2. Cameroon. *Agri-stat: Annuaire des statistiques du secteur agricole*, 17th ed.; National government: Yaoundé, Cameroon, 2012; pp. 1-123.

3. Desneux, N.; Luna, M.; Guillemaud, T.; Urbaneja, A. The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production. *J Pestic Sci* **2011**, *84*, 403–8.

4. Samira, G.C.; Habib, A. Chemical composition and insecticidal effects of the essential oil of cardamom, *Elettaria cardamomum* on the tomato leaf miner, Tuta absoluta. *Toxin Rev.* **2017**, *36*, 12–17.

5. Zekeya, N.; Chacha, M.; Ndakidemi, P.A.; Materu, C.; Chidege, M.; Mbega, E.R. Tomato leafminer (*Tuta absoluta* Meyrick, 1917): A threat to Tomato production in Africa. *JAERI* **2017,** *1*, 1-10.

6. Mohamed, E.S.I.; Mohamed, M.E.; Gamiel, S.A. First record of the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Suda. *Bulletin OEPP/EPPO* **2012**, *42*, 325-327.

7. Gilardon, E.; Pocovi, M.; Hernandez, C.; Collavino, G.; Olsen A. Role of 2-tridecanone and Type VI glandular trichome on tomato resistance to *Tuta absoluta*. *Pesqui. Agropecu. Bras.* **2001**, *36*, 929–933.

8. Tak, J-H.; Isman, M.B. Metabolism of citral, the major constituent of lemongrass oil, in the cabbage looper, *Trichoplusia ni*, and effects of enzyme inhibitors on toxicity and metabolism. *Pest Biochem Physiol* **2016**, 1-30.

9. Regnault-Roger, C. The potential of botanical essential oils for insect pest control. *Integrated Pest Manag. Rev.* **1997**, *2*, 25–34.

10. Krčmar, S.; Gvozdić, V. Field studies of the efficacy of some commercially available essential oils against horse fies (Diptera: Tabanidae). *Entomol. Gen.* **2016**, *36*, 97–105.

11. Campolo, O.; Malacrinò, A.; Zappalà, L.; Laudani, F.; Chiera, E.; Serra, D.; Russo, M.; Palmeri, V. Fumigant bioactivity of five Citrus essential oils against *Tribolium confusum*. *Phytoparasitica* **2014**, *42*, 223–233.

12. Hernández-Lambraño, R.; Caballero-Gallardo, k.; Olivero-Verbel, J. Toxicity and antifeedant activity of essential oils from three aromatic plants grown in Colombia against *Euprosterna elaeasa* and *Acharia fusca* (Lepidoptera: Limacodidae). *Asian Pac. J. Trop. Biomed.* **2014**, *4*, 695-700.

13. Slimane, B.B.; Ezzine, O.; Dhahri, S.; Jamaa, M.L.B. (2014). Essential oils from two Eucalyptus from Tunisia and their insecticidal action on *Orgyia trigotephras* (Lepidotera, Lymantriidae). *BR* **2014**, 47, 1-8.

14. Szczepanik, M.; Zawitowska, B.; Szumny, A. Insecticidal activities of *Thymus vulgaris* essential oil and its components (thymol and carvacrol) against larvae of lesser mealworm, *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae). *Allelopathy J.* **2012**, 30, 129-142.

15. Veras, S.S.; Zambrano, D.F.; Méndez-Sanchez, S.C.; Rodríguez-Sanabria, F.; Stashenko, E.E.; Luna, J.E.D. Essential oils with insecticidal activity against larvae of *Aedes aegypti* (Diptera: Culicidae). *Parasitol. Res.* **2014**, *113*, 2647–2654.

16. Sammour, E.A.; Kandil, M.A-H.; Abdel-Aziz, N.F.; El Maguied Agamy, E.A.; El-Bakry, A.M.; Abdelmaksoud, N.M. “Field evaluation of new formulation types of essential oils against *Tuta absoluta* and their side effects on Tomato plants". *ASAG* **2018**, *2*, 15-22.

17. Simo Kamdem, M.; Sameza M.L.; Jazet Dongmo, P.M.; Fekam Boyom, F.; Bakargna-via, I.; Hzounda Fokou, J-B.; Kenfack Tsague, I.F.; Zeuko’o Menkem, E.; Amvam Zollo, P.H.; Menut, C. Antiradical, Anti-inflammatory and Antifungal Activities of essential oils of Two Aromatic Plants: *Apium graveolens* (Apiaceae) and *Thymus vulgaris* (Lamiaceae), *J. Life Sci.* **2015**, *9*, 51-64.

18. Nguefack, J.; Dongmo, J.B.; Dakole, C.D.; Leth, V.; Vismer, H.F.; Torp, J.; Guemdjom, E.F.N.; Mbeffo, M.; Tamgue, O.; Fotio, D.; Amvam Zollo, P.H.; Nkengfack, A.E. “Food preservative potential of essential oils and fractions from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* against mycotoxigenic Fungi.” *Int. J. Food Microbiol.* **2009**, *131*, 151-156.

19. Tchinda, E.S.; Jazet, P.M.D.; Tatsadjieu, L.N.; Ndongson, B.D.; Amvam P.H.Z.; Menut C.. Antifungal activity of the essential Oil of *Cymbopogon citratus* (Poaceae) Against *Phaeoramularia angolensis*, *J. Essent. Oil Bear. Pl.* **2009**, *12*, 218-224.

20. Braga, M.E.M.; Ehlert, P.A.D.; Ming, L.C.; Meieles, M.A.A. Super critical fluid extraction from lippia alba: global yields, kinetic data and extract chemical composition. *J. Supercrit. Fluids* **2005**, *34*, 149–156.

21. Tak, J-H.; Jovel, E.; Isman, M.B. Contact, fumigant, and cytotoxic activities of thyme and lemongrass essential oils against larvae and an ovarian cell line of the cabbage *Trichoplusia ni*. *J. Pest Sci.* **2015**, *140*, 9-16.

22. Roser, V.; Blanca, F.; Salvador, C. Antifungal compounds from plants. *Rec. Adv. Pharm. Sci.* **2013**, *3*, 23–43.

23. El-Mesallamy, A.M.; Raslan, S.A.; EL-Nagar, M.E.; El-Medany, W.A.Z. Toxicological and Biological effect of *Ocimum basilicum* L. oil on some Cotton Pests. *Middle East J. Agric. Res.* **2015**, *4*, 949-955.

24. Biondi, A.; Zappalà, L.; Stark, J.; Desneux, N. Do biopesticides affect the demographic traits of a parasitoid wasp and its biocontrol services through sublethal effects? *PLoS One* **2013**, *8*, 1-11.

 doi:10.20944/preprints201909.0121.v1

25. Campolo, O.; Cherif, A.; Ricupero, M.; Siscaro, G.; Grissa-Lebdi, K.; Russo, A.; Cucci, L.M.; Di Pietro, P.; Satriano, C.; Desneux, N.; Biondi, A.; Zappalà, L.; Palmeri, V. Citrus peel essential oil nanoformulations to control the tomato borer, *Tuta absoluta*: chemical properties and biological activity. Scientific Reports, *NPG* **2017**, *7*, 1-11.

26. Benchouikh, A.; Allam, T.; Abderrahim, K.; Khadija, O. The study of the insecticidal effect of *nigella sativa* essential oil against *tuta absoluta* larvae. *IJSTR* **2015**, *4*, 88-90.

27. Sawicki, R.M. Insecticidal activity of pyrethrum extract and its four insecticidal constituents against house flies. Knock-down and recovery of flies treated with pyrethrum extract with and without piperonyl butoxide. *J. Sci. Food Agric.* **1962**, *13*, 283-291.

28. Molan, A-L.; Rathi, M.H.; Abdulwahab, D.A. Larvicidal and pupicidal activity of water extracts from tomato pomaces and their components against *culex quinquefasciatus* (diptera: culicidae) under laboratory conditions. *WJPPS* **2016**, *5*, 163-171.