

*Full Length Research Paper*

# **Chemical composition and bioactivity of *Lantana camara* L. essential oils from diverse climatic zones of Kenya against leaf miner (*Tuta absoluta* Meyrick)**

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**In recent years, essential oils (EOs) as alternatives to synthetic pesticides in managing pests have been assessed. The use of bioinsecticide in pest management is encouraged in agroecology for a sustainable agricultural system. Essential oils of *Lantana camara* L. leaves from different climatic zones of Kenya were extracted by steam distillation and analyzed through GC-MS to identify the compounds. The contact toxicity and repellent activity of EOs against the invasive tomato pest, *Tuta absoluta*, were tested. The toxicological assays were performed following the leaf-dip bioassay protocol, while the repellency activity was performed using the repellent response method for phytophagous pests and the data analyzed using the ANOVA test. It was found that *L. camara* EO has a good insecticidal activity with higher mortality (89%) on the 2<sup>nd</sup> instar larvae with a higher dosage (0.01 µl/µl). The repellence test also showed a higher average repellence (93.44%) effect with a higher dosage (0.01 µl/µl) of the EOs. According to these results, the EO of *L. camara* may well be a sustainable, eco-friendly alternative for synthetic insecticide in the *T. absoluta* management program.**

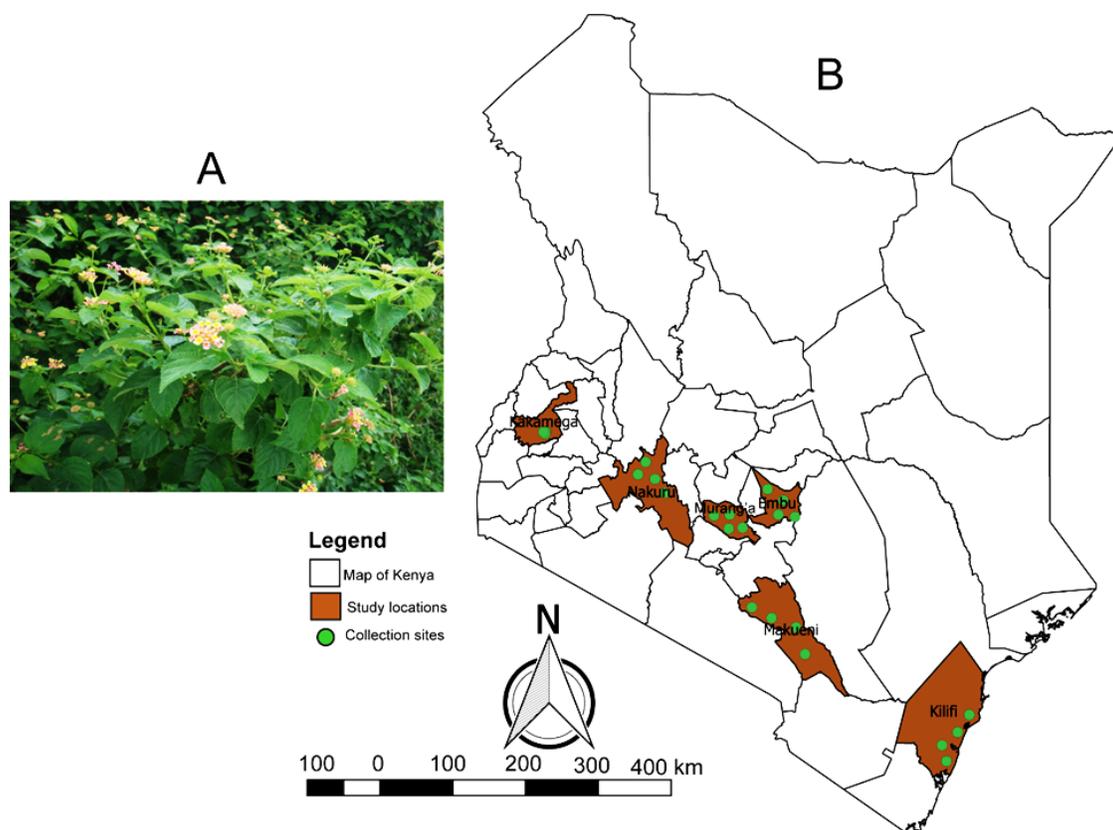
**Key words:** Bioinsecticide; essential oil, integrated pest management, invasive species, secondary metabolites.

## **INTRODUCTION**

The leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a pest of vital global economic importance. It limits tomato production (*Lycopersicon esculentum* L.) worldwide (Campos et al., 2017; Desneux et al., 2011; Guedes et al., 2019). As a result, this pest has gained notoriety as a pest species that can cause destruction and losses of up to 100% when there is no intervention (Rwomushana et al., 2019).

Current management of *T. absoluta* typically relies solely on synthetic insecticides (Silva et al., 2019). However, this management strategy has not provided a total solution to the problem due to insecticide resistance and pest resurgence (Guedes et al., 2019; Roditakis et al., 2018). Furthermore, these products are already proving to be harmful to the environment (Damalas and Koutroubas, 2018; Gill and Harsh, 2014), causing the

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**Figure 1.** (A) Morphology of fresh leaves of *L. camara* (B) The collection sites from six climatic zones of Kenya.

development and progression of several health issues in humans (Özkara et al., 2016). To cut back the excessive use of synthetic insecticides, exploring environmentally friendly and sustainable alternative strategies, such as natural products from wild plants, remains a viable option. Natural products directly utilized as pest control agents offer a more sustainable pest management solution than synthetic pesticides (Nuruzzaman et al., 2019).

Essential oils (EOs) from pesticidal plants used as pesticides play a crucial role in controlling pests sustainably (Campolo et al., 2017) by reducing the pest population while minimizing the environmental effect. *Lantana camara* is a wild plant that produces Eos, extensively studied for its bioactive properties and reported as having insecticidal (Javier et al., 2017; Murugesan et al., 2012, 2016), acaricidal (Adehan et al., 2016), larvicidal (Costa et al., 2010; Zandi-Sohani et al., 2012), fumigant (Gotyal et al., 2016), acetylcholine inhibition, repellent (Yuan and Hu, 2012), and antifeedant (Chau et al., 2019; Yuan and Hu, 2012) action among other features against a wide range of pests. These bioactive properties exhibited by *L. camara* EO make it a novel candidate for use as a pesticide with multiple actions. For example, Murugesan et al. (2016) tested the *L. camara* EO on *Hyblaea puera* and *Ahevidae fabriciella* (Lepidoptera) at a concentration of 10000 ppm,

reported a 62% larval mortality and concluded that the EO expressed insecticidal and antifeedant properties. Besides, Javier et al. (2017) tested the EO of *L. camara* for bioactivity against *Spodoptera litura* (Lepidoptera), showing remarkable insect growth regulatory activities and direct toxicity. This corroborates Deshmukhe et al. (2011) that it has the potential to be exploited as a botanical insecticide for cutworm management. In their study, Costa et al. (2010) tested for larvicidal activity against *A. aegypti* larvae using the EOs from the leaves of *L. camara* and showed that it has larvicidal potential. However, all these studies show the potential this plant has in the management of pests. There is no scientific investigation of its EO bioactivity on *T. absoluta*.

## MATERIALS AND METHODS

### Plant materials

Fresh, healthy first four leaves of the stem from the top of *L. camara* plants (Figure 1A) were collected from six different climatic zones of Kenya, namely; Lower Highland-Nakuru (LH-NJ), Upper Midland 1-Kakamega (UM1-KK), Upper Midland 2-Kandara, Murang'a (UM2-KA), Upper Midland 3-Embu (UM3-EM), Lower Midland-Kiboko, Makeni (LM-KI) and Coastal Lowland-Mtwapa, Kilifi (CL-MT) in July 2018. The exact location of the collection sites are marked with dots in Figure 1B. The leaves of *L. camara* from each sampling site

were harvested from the first four leaves of the stem from the top of plants and mixed to make a composite sample. The samples were transferred to the lab within two days of collection in ventilated nylon gunny bags. The samples were washed and then air-dried immediately under room temperature (23–26°C) in a well-ventilated room for two weeks until crisp. Subsequently, they were grounded into powder and stored in khaki bags until it was time for oil extraction.

### Extraction of the essential oils

The *L. camara* EO was isolated by steam distillation using a steam distiller apparatus (Deschem Science supply, China). First, 200 g of dried leaves from each sample was steam distilled separately using 2000 mL distilled water for 3 h. Next, the condensing oils were separated with a separating funnel, and the oily sample was treated with anhydrous sodium sulphate ( $\text{Na}_2\text{SO}_4$ ) to remove the remaining trace of water and collected in amber-coloured vials, labelled, and stored at 4°C until use. The experiment was performed in triplicates for each sample, and the yield was averaged over triplicates. The percentage yields of EOs were determined based on dry matter and calculated as the weight of the extracted oil (g) /200 g of the dry weight of the leaf sample (% w/w).

### Gas chromatography-mass spectrometry (GC-MS) analysis

The EO samples were diluted in n-hexane and transferred to the auto-sampler vials for GC-MS analysis. GC-MS-QP2010 SE (Shimadzu Corporation) coupled with an autosampler was used for this study. Ultrapure He was used as the carrier gas at a flow rate of 1 ml/min. A BPX5 non-polar column, 30 m; 0.25 mm ID; 0.25 µm film thickness, was used for separation. The GC was programmed as follows: 60°C (1 min); 10°C/min to 250°C (25 min). The total run-time was 45 min. Only 1 µL of the sample was injected. The injection was done at 200°C in split mode, with a divided ratio set to 10:1 and the interface temperature set at 250°C. The Electron Ionization (EI) ion source was set at 200°C. Simultaneously, the mass analysis was done in full scan mode, in a range of 40 – 550 amu (Shimadzu GC-MS QP2010 SE solution software (Tokyo, Japan)). The raw mass spectra obtained matched against the NIST 2017 Library of Mass Spectra for possible identification of compounds. Samples were analyzed at the Jomo Kenyatta University of Agriculture and Technology Analytical Chemistry Laboratory.

### Rearing of test insects and plants

*T. absoluta* insects for the experiment originated from infested tomato leaves collected at the International Centre of Insect Physiology and Ecology (ICIPE) (S01°13.140'; E036°53.440') Insectary Laboratory, Nairobi, Kenya. The adults were transferred to the laboratory within 2 h. Upon arrival in the laboratory, they were released in the insect-proof rearing cages (50×60×80 cm) and provided with four insect-free four potted tomato plants (height: 25 cm) and reared to the first filial (F1) generation as described in Roditakis et al. (2013). Newly-emerged adults of *T. absoluta* (F1 generation) were released inside another net cage to obtain homogeneous *T. absoluta* larvae (same age, nutritional and general health) (IRAC method No. 022). The adults of *T. absoluta* were provided with water and an energy source (commercial honey 1:1 dilution). The insects were allowed to oviposit for 24–48 h. The oviposition level was evaluated visually. If an adequate number of eggs were observed (that is, more than 150–200 eggs), then the plant material was carefully removed, and new plants were placed in the oviposition area to allow the continuation of the oviposition

(IRAC method No. 022). The plant material infested with *T. absoluta* eggs were placed in an insect-proof rearing cage to allow larval development to the second instar.

Tomato (Rio Grande VF) plants (*L. esculentum*) used for rearing *T. absoluta* and for performing the bioassays experiments were grown in the greenhouse conditions in 2-L pots, inside large insect-proof cages and maintained pest-free under (30±3°C) temperature, (75-80%) relative humidity and (12:12) light: dark conditions. The plants were screened for the presence of pests every second day; in the rare event of detected infestations, these were manually removed by cutting the infested leaves or removing and destroying the plant (Roditakis et al., 2013). Therefore, no insecticides were used during the plant development phase.

### Bioassay experiments

The experiments were conducted at the Department of Horticulture and Food Security at Jomo Kenyatta University of Agriculture and Technology (Kenya) under controlled environmental conditions in growth chambers, maintaining a temperature of 25 ±2°C, 65-70% relative humidity, and 12:12 light: dark photoperiod regime.

### Contact toxicity

The second instar larvae were collected from the rearing cages, and accordingly, the toxicological assays were performed following the leaf-dip bioassay protocol of the 199 Insecticide Resistance Action Committee (IRAC) test method 022 ([www.irac-online.org](http://www.irac-online.org)), with minor modifications. The EOs extracted from plants from the six climatic zones were prepared in three different dosages (0.01, 0.001, and 0.0001 µl/µl) mixed with 0.1% Tween® 20 (Sigma-Aldrich, Germany) and used as treatments. A commercial formulation of Flubendiamide (BELT® 480 SC, Bayer AG, Germany) insecticide was used as a positive control, whereas sterile distilled water containing 0.1% Tween® 20 (Sigma-Aldrich, Germany) as a non-ionic wetting agent was used as a negative control with three replications. The insecticide was used as per the manufacturer's recommendations (0.1 µl/µl).

Sufficient non-infested, untreated tender young whole tomato leaflets of uniform size were collected and kept in sealed plastic bags to prevent them from wilting. Complete tomato leaflets were dipped for 5 s in the EO concentrations with gentle agitation to ensure the entire surface is covered equally. The treated leaflets were dried on a wire net with an upper leaf surface (abaxial surface) facing skywards and placed in a labelled petri dish (Ø- 90 mm) with slightly moistened filter paper covering the bottom. Around 0.2 ml distilled water was used; which was sufficient to wet the filter paper keeping the leaf material turgid throughout the bioassay period. Second-instar *T. absoluta* larvae were carefully removed from the galleries in infested tomato leaves under a light-bed (transparent bench with fluorescent illumination underneath). In each petri dish, ten larvae (2<sup>nd</sup> instar) (4-5 mm) were released carefully using a subtle soft brush to avoid damaging the very fragile larvae. Subsequently, all the Petri dishes sealed using a ventilated muslin cloth.

Larval mortality was assessed after 24, 48, 72, and 96 h of exposure whereas death was recorded under a magnifying glass (Osho® 10x magnification, Kenya). The larvae were scored as dead if they could not make coordinated movement from a gentle stimulus with a fine brush to the posterior body segment. The experiment was repeated three times simultaneously, and the average mortality obtained.

### Repellent activity

In testing the repellent activity of the EOs, Whatman No. 1 filter

paper was cut to fit the size of the plastic dish and divided into two equal parts. Half of the filter papers was treated with 0.1% Tween® 20 as control and the other half with EO concentrations mixed with 0.1% Tween® 20 of 0.01, 0.001, and 0.0001 µl/µl. Twenty larvae (2<sup>nd</sup> instar) were placed in the middle of each Petri dish. The plastic dishes were closed and tightened with Parafilm. The experiment was carried out in five replications and at the same environmental conditions with insect rearing. After four hours of exposure, the number of insects in each half of the filter paper was recorded and the percentage repellency (PR) calculated using the following formula:

$$PR = [(NC - NT)/(NC + NT)] \times 100 \quad (1)$$

PR = percentage repellency, NC = number of larvae in the control area and NT = number of larvae in the treatment area. The mean repellency value of each extract was calculated and assigned to repellency classes from 0 to V: class 0 (PR ≤ 0.1%), class I (PR = 0.1-20%), class II (PR = 20.1-40%), class III (40.1-60%), class IV (60.1-80%), and class V (80.1-100%).

### Statistical analysis

The mean number of live larvae per leaf was tested for per cent mortality. The per cent mortalities were corrected for control (that is, natural) mortality using Abbott's formula (Abbott, 1925). The two-way analysis was conducted with the EOs from different climatic zones as the main effect, concentration rate as the covariate, and larvae mortality registered at different time intervals (24, 48, 72, and 96 h) as the response variable. In addition, the toxicity effect of the different EOs concentration on the second instar larvae was compared using the analysis of variance (two-way ANOVA) and the means compared by LSD test at 5% level (SAS®, On-Demand for Academics).

Concentration–mortality data (obtained from the bioassay) was computed using Probit procedure (PROC PROBIT LOG10; SAS®, On-Demand for Academics.) to estimate the median lethal concentrations (LC<sub>50</sub> values), their 95% fiducial limits (FL) along with their respective standard errors, as well as slopes of the curves. The LC<sub>50</sub> values were significantly different when their 95% fiducial limits did not show similarity.

Per cent of larval repellency was analyzed using ANOVA (Analysis of variance) (SAS®, On-Demand for Academics). The negative values were treated as zero. The larval repellency was calculated using the simplified contact repellency test. The mean percentage of larvae that repelled into the untreated side was corrected by a control test. The square root of the per cent of repellency in each test was arcsin converted, and ANOVA and the multiple comparisons of the repellency by LSD was performed using SAS®, On-Demand for Academics.

## RESULTS

### Chemical composition and content of *Lantana camara* L. essential oils

The GC-MS analysis of *L. camara* EO from the same species growing in diverse climatic zones of Kenya resulted in 123 compounds with a higher content than 0.01%, of the oils. Sesquiterpenes followed by monoterpenes dominated the significant components identified (over 1.0% in content) (Table 1) listed in order of their elution on the HP-5 MS column. The

sesquiterpenes were dominated by the β-caryophyllene (5.11 - 14.31%) and spathulenol (4.22 - 9.50%) as sesquiterpene hydrocarbon and oxygenated monoterpene, respectively. On the other hand, the monoterpenes were dominated by Sabinene (2.84 - 12.54%) and eucalyptol (1.44 - 8.81%) as monoterpene hydrocarbon and oxygenated monoterpene, respectively.

The yield of the essential oil of *L. camara* collected at different regions was obtained from dried leaves that ranged from 0.25 to 0.37% w/w. The highest yield of oil was observed in samples collected from Mtwapa (CL-MT) (0.37%), characterized by high mean temperatures (26°C) and average precipitation of 125 mm. The opposite result was observed for the oil obtained from samples collected in Kakamega, which presented a lower yield of 0.25%. During this period, there was lower maximum (24.5°C) and minimum (15°C) temperatures, in addition to higher volume of total precipitation (283 mm). In terms of the compounds, the samples from Kakamega exhibited higher monoterpenes levels, with Sabinene being the predominant compound, while the samples from Mtwapa exhibited higher levels of Sesquiterpenes with β-caryophyllene being the predominant compound.

### Toxicological bioassays

The toxicological two-way analysis of variance suggests that both the dose concentration rate ( $F = 13.11$ ;  $df = 2$ ;  $p < 0.0001$ ) and the EO formulations ( $F = 6.44$ ;  $df = 7$ ;  $p < 0.0001$ ), registered at 24 h, 48 h, 72 h and 96 h after the treatment influenced the larva mortality. The statistical analysis revealed a significant interaction between dose concentration, EO formulations and observation time ( $p < 0.0001$ ). However, there was no interaction among the two variables, the dose concentration and EOs formulations ( $F = 0.01$ ;  $df = 10$ ;  $p 1.00$ ).

### Contact toxicity on larvae

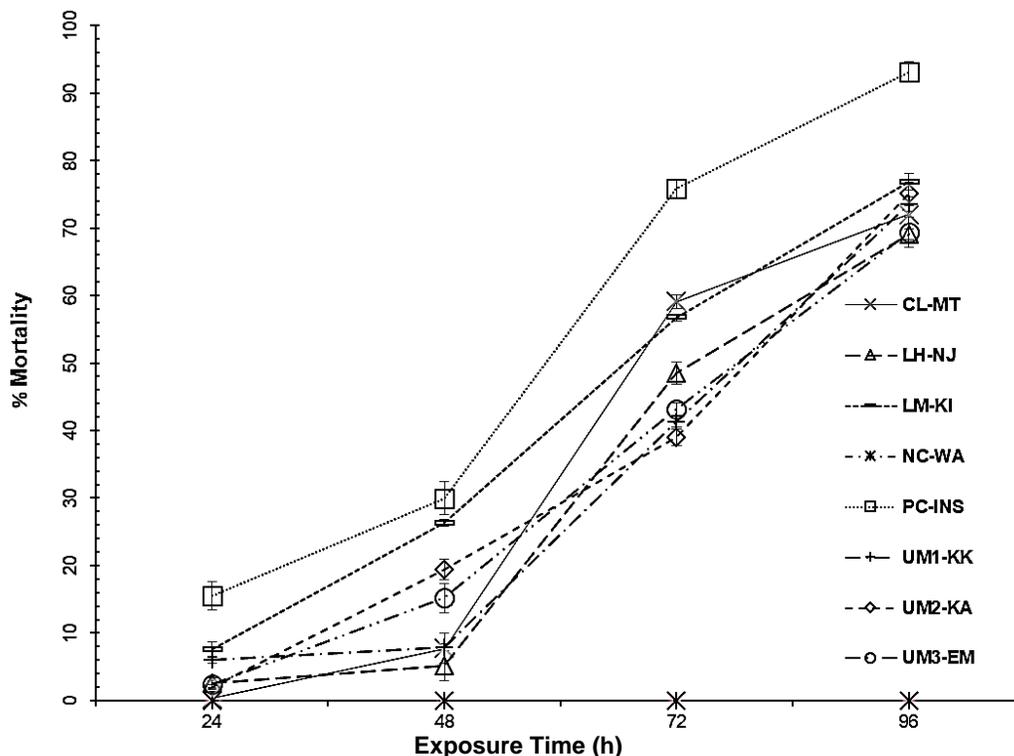
The mean average larval mortality in the positive control-insecticide -Flubendiamide) (PC-INS) was 16, 30, 76 and 93% after 24, 48, 72 and 96 h of exposure, respectively. Whereas in the negative control-water (NC-WA) treatment, no larvae died during the 96 h of the experiment. The positive control (PC-INS) showed the highest larval mortality compared to the EOs formulations (Figure 2).

In the first sampling (24 h after the treatment), LM-KI and UM1-KK EO formulations were the most effective in killing the larvae's 8 and 6% mortality, respectively. Overall, the positive control (PC-INS), on average, was most effective than the EO formulations (Figure 2). In the second sampling (48 h), the mortality of *T. absoluta* larvae increased significantly in all the treatments compared to the first sampling. In the third, and fourth

**Table 1.** Composition of EO of the leaves of *L. camara* from diverse climatic zones of Kenya.

S/N	Name of compound <sup>a</sup>	RI	RT	UM1-KK (%) <sup>c</sup>	LM-KI (%) <sup>c</sup>	CL-MT (%) <sup>c</sup>	LH-NJ (%) <sup>c</sup>	UM3-EM (%) <sup>c</sup>	UM2-KA (%) <sup>c</sup>
1	$\alpha$ -Pinene	1063	5.13	1.15	1.02	-	-	3.66	2.16
2	Camphene	1086	5.45	-	-	-	-	1.9	1.72
3	Sabinene	1107	5.78	<b>12.54</b>	<b>5.79</b>	<b>4.31</b>	2.84	<b>9.66</b>	3.19
4	Cyclopentene, 3-isopropenyl-5,5-dimethyl-	1107	5.79	-	-	-	-	-	3.22
5	$\beta$ -Pinene	1115	5.95	-	-	-	-	3.33	2.24
6	3-Carene	1138	6.39	-	1.02	-	-	3.24	2.28
7	Isosylvestrene	1154	6.7	1.02	1.08	-	-	-	1.94
8	$\beta$ -Ocimene	1157	6.75	-	-	-	-	<b>4.06</b>	-
9	Eucalyptol	1160	6.81	<b>8.81</b>	3.72	2.97	1.44	<b>6.52</b>	2.96
10	cis-Sabinene hydrate	1191	7.42	1.29	-	-	-	1.3	-
11	Linalool	1210	7.79	-	-	-	-	1.04	-
12	Camphor	1264	8.84	2.32	1.02	-	-	-	-
13	(+)-2-Bornanone	1265	8.86	-	-	-	-	2.19	1.11
14	L-4-terpineneol	1287	9.29	1.52	-	-	-	1.08	-
15	L- $\alpha$ -Terpineol	1299	9.52	1.52	1.51	-	-	1.85	-
16	Guaia-10(14)	1468	12.35	-	-	1.32	1.57	-	-
17	$\beta$ -Elemene	1468	12.36	-	1.52	-	-	1.84	2.86
18	$\beta$ -Caryophyllene	1506	12.95	<b>11.65</b>	<b>10.52</b>	<b>12.45</b>	<b>14.31</b>	<b>8.78</b>	<b>5.11</b>
19	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	1545	13.5	<b>5.77</b>	<b>7.41</b>	<b>7.68</b>	<b>8.53</b>	<b>5.11</b>	<b>6.23</b>
20	$\gamma$ -Murolene	1554	13.62	-	1.03	-	1.1	1.24	2.43
21	Germacrene D	1564	13.76	1.82	-	-	-	-	-
22	$\beta$ -copaene	1565	13.78	-	2.93	2.61	5.18	3.28	5.92
23	Davana ether	1571	13.86	-	<b>4.57</b>	<b>4.65</b>	3.33	1.8	2.34
24	Bicylogermacrene	1579	13.98	<b>6.94</b>	<b>6.29</b>	<b>6.73</b>	<b>10.05</b>	<b>6.93</b>	2.15
25	$\alpha$ -Murolene	1582	14.02	-	-	-	-	-	<b>4.96</b>
26	$\alpha$ -cadinene	1593	14.18	-	1.19	1.19	1.61	-	1.52
27	Cubebol	1597	14.23	-	1.23	1.16	1.35	-	1.16
28	(E)-Nerolidol	1627	14.63	<b>8.36</b>	<b>7.01</b>	<b>7.16</b>	<b>5.55</b>	3.63	<b>4.77</b>
29	Davanone	1647	14.88	2.05	1.68	1.68	1.17	-	-
30	Spathulenol	1668	15.16	<b>7.54</b>	<b>6.67</b>	<b>7.53</b>	<b>9.5</b>	<b>4.22</b>	<b>6.19</b>
31	Caryophyllene oxide	1674	15.24	<b>5.51</b>	<b>5.06</b>	<b>5.64</b>	<b>4.29</b>	3.43	2.69
32	$\alpha$ -Humulene 1,2 epoxide	1702	15.6	-	3.31	3.54	2.86	1.44	2.34
33	Isospathulenol	1723	15.85	-	2	1	-	-	1.16
34	Thujene	1728	15.91	<b>4.66</b>	<b>5.45</b>	<b>6.33</b>	<b>5.17</b>	2.25	-
35	Humulane-1,6-dien-3-ol	1729	15.92	-	-	-	-	-	3.18
36	Eudesma-4(15),7-dien-1.beta.-ol	1745	16.11	1.88	1.4	1.44	1.48	-	1.39
37	trans-Chrysanthenol	1764	16.35	3.89	-	-	1.11	-	-
38	Isocaryophyllene	1995	19.96	-	-	-	-	-	4.81
	<b>Total % Area</b>			<b>90.24</b>	<b>84.43</b>	<b>79.39</b>	<b>82.44</b>	<b>83.78</b>	<b>82.03</b>
	Monoterpene hydrocarbons			18.35	13.28	10.64	8.01	28.1	11.59
	Oxygenated monoterpenes			3.04	1.51	0	0	6.16	1.11
	Sesquiterpene hydrocarbons			25.07	33.47	34.01	41.63	25.76	33.26
	Oxygenated sesquiterpenes			25.34	23.82	25.77	23.56	11.28	19.38
	Others			18.44	12.35	8.97	9.24	12.48	16.69
	Essential oil yield % (v/w) <sup>b</sup>			0.25	0.3	0.37	0.35	0.35	0.35

RT, Retention Time. <sup>a</sup> Compounds identification based on data obtained from the NIST 2017 Library of the Gas Chromatography-Mass Spectrometry System. <sup>b</sup> Yield of isolated oils expressed as mL of essential oil/200 g of dry leaves. Bold values signify the significant components of the essential oils. <sup>c</sup> Relative percentage '-' not-detected. UM1-KK-Upper Midland 1-Kakamega, LM-KI- Lower Midland-Kiboko, CL-MT- Coastal Lowland-Mtwapa, LH-NJ- Lower Highland-Njoro, UM3-EM- Upper Midland 3-Embu, UM2-KA- Upper Midland 2-Kandara.



**Figure 2.** Mean mortality of *Tuta absoluta* larvae exposed to different EOs formulations and the two controls ( $\pm$  control) in the toxicological bioassay. *Lantana* oil treatments were sampled from different climatic zones- UM1-KK-Upper Midland 1-Kakamega, LM-KI- Lower Midland-Kiboko, CL-MT- Coastal Lowland-Mtwapa, LH-NJ- Lower Highland-Njoro, UM3-EM- Upper Midland 3-Embu, UM2-KA- Upper Midland 2-Kandara, and the control- PC-INS- positive control-insecticide –Flubendiamide, NC-WA- negative control-water.

sampling, the mortality rate increased for all the treatments, each attaining above 40% larval mortality except UM-KA, treatment which was 39%. In the fourth sampling, all the treatments reached a mortality rate above 60%.

Throughout the trial, the LM-KI EO formulation was the most effective against the larvae (maximum average mortality = 77%), whereas the LH-NJ and UM3-EM EO formulation could only kill a maximum of 69% of the exposed larvae. Overall, there was no significant difference in the mortality induction by the different EO formulations.

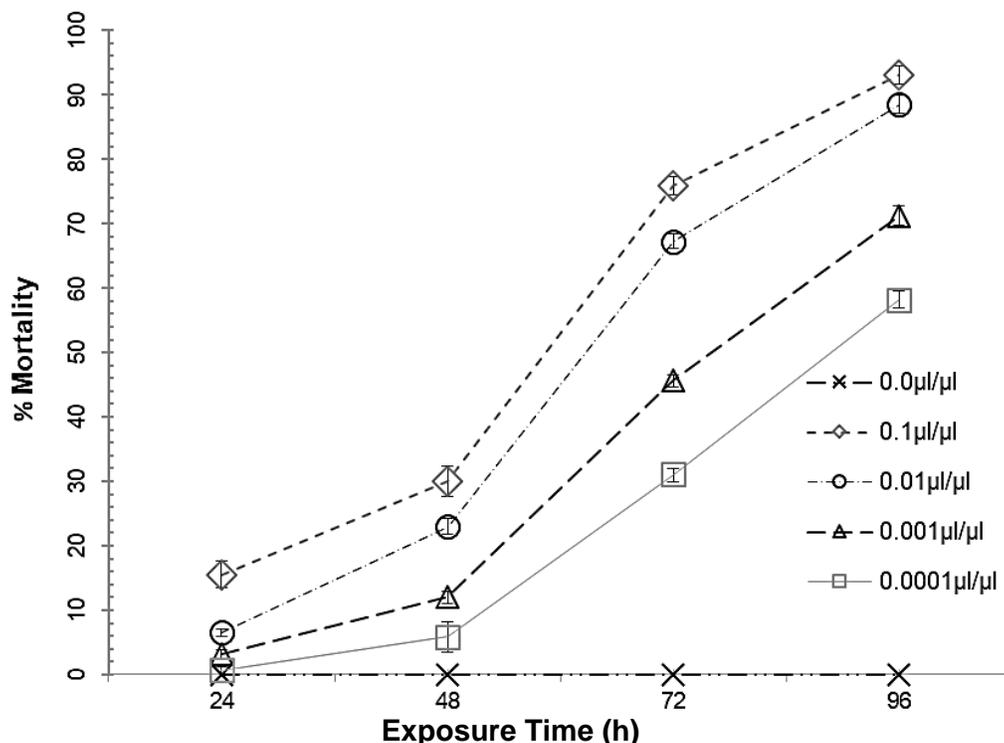
The dose concentration profoundly influenced the larval mortality rate. In all the time point, the positive control (0.1  $\mu\text{l}/\mu\text{l}$ -insecticide recommended dosage), on average, was most effective in killing the larvae's (average mortality= 89%) than the EO formulations dosage concentrations of 0.01, 0.001, and 0.0001  $\mu\text{l}/\mu\text{l}$  (average mortality= 89, 71 and 58% respectively) (Figure 3). The longer the larvae's exposure to the treatment, the more the mortality rate increased in all the dose concentration. The highest dosage of the EO formulations (0.01  $\mu\text{l}/\mu\text{l}$ ) showed the highest mortality at all the time point of sampling, which was reasonably comparable to the

positive control (0.1  $\mu\text{l}/\mu\text{l}$ ).

The EO concentrations of 0.1  $\mu\text{l}/\mu\text{l}$  were the most effective in killing the larvae at 16% mortality, followed by 0.01  $\mu\text{l}/\mu\text{l}$  at 7% in the first sampling (24 h after the treatment). The mortality rate of *T. absoluta* larvae increased in all the treatments in the second sampling (48 h), and in the 0.01  $\mu\text{l}/\mu\text{l}$  dose concentration, the mortality rates increased three times, compared to the first sampling. None of the dosage concentration, including the positive control, attained 50% larval mortality within the early 48 h of larval exposure. However, by 72 h, the positive and 0.01  $\mu\text{l}/\mu\text{l}$  dosage concentration had achieved above 50% mortality.

#### **Median lethal concentrations ( $LC_{50}$ ) for the larvae**

The  $LC_{50}$  values (Table 2) was calculated for 72 h because the maximum mortality registered at 24 and 48 h were less than 50%, while at 96 h, it was over 50%. Therefore, this was the best time since the longer the exposure time, the lower the  $LC_{50}$  values. The dose-response mortality data exposed to the EO formulations presented  $\chi^2$  values < 34.33. This parameter shows the



**Figure 3.** Mean mortality of *Tuta absoluta* larvae exposed to different EO concentration rates and the controls in the toxicological bioassay. 0.1 µl/µl- positive control-insecticide recommended dosage, 0.0 µl/µl- negative control- water 0.01, 0.001, and 0.0001 µl/µl - EO formulations dosage concentrations.

**Table 2.** Estimated median lethal concentrations (LC<sub>50</sub>) of the different EOs of *L. camara* on *T. absoluta* larvae at 72 hours in the toxicity test on larvae bioassays.

Essential oil	LC <sub>50</sub> (µl/µl)	95% of Fiducial limits	Slope	Intercept ± SE	χ <sup>2</sup> (df = 1)
CL-MT	0.00025 <sup>a</sup>	0.000059-0.00059	2.45	1.47±0.292	25.32 <sup>s</sup>
LH-NJ	0.00123 <sup>b</sup>	0.00056-0.0029	2.24	1.30±0.285	20.83 <sup>s</sup>
LM-KI	0.00044 <sup>a</sup>	0.00018-0.00085	1.94	1.73±0.295	34.33 <sup>s</sup>
UM1-KK	0.00246 <sup>c</sup>	0.00144-0.00478	0.94	2.79±0.492	11.97 <sup>s</sup>
UM2-KA	0.00542 <sup>d</sup>	0.00222-0.0296	2.49	0.91±0.281	10.31 <sup>s</sup>
UM3-EM	0.00242 <sup>c</sup>	0.001021-0.00621	1.26	2.07±0.468	19.61 <sup>s</sup>

Different letters within the same column of each trial indicate statistical differences ( $p < 0.05$ ); s = significant ( $\alpha > 0.05$ ), df –degree of freedom, SE- standard error. CL-MT- Coastal Lowland-Mtwapa, LH-NJ- Lower Highland-Njoro, LM-KI- Lower Midland-Kiboko, UM1-KK-Upper Midland 1-Kakamega, UM2-KA- Upper Midland 2-Kandara, UM3-EM- Upper Midland 3-Embu.

appropriateness of the model to estimate the LC<sub>50</sub>. Evaluation of toxicity of the EOs, based on LC<sub>50</sub> values and fiducial limits, showed that significant differences among- EO toxicity for CL-MT, LH-NJ, LM-KI, UM1-KK, UM2-KA and UM3-EM were 0.00025, 0.00123, 0.00044, 0.00246, 0.00542 and 0.00242 µl/µl, respectively (Table 2). The EO extracted from CL-MT climatic zone showed the highest capacity to kill the exposed larvae (LC<sub>50</sub> = 0.00025 µl/µl). The EO from the UM2-KA formulation required the highest concentration (LC<sub>50</sub> = 0.00542 µl/µl)

to kill 50% of the exposed larvae (Table 2). The mortality rate of the larvae depended on the concentration of EO and increased with increasing oil dose concentrations. For all the six oil formulations, the highest average mortality (93.44%) was observed at the highest dose concentration (0.01 µl/µl) (Figure 3).

### Repellent activity

Results given in Table 3 describes the repellent activity of

**Table 3.** Repellent activity of different dose concentrations of EOs from *L. camara* leaves against *T. absoluta* larvae.

EOs	CL-MT	LH-NJ	LM-KI	UM1-KK	UM2-KA	UM3-EM	Class
Dose (0.1 µl/µl)	Repellency (%) ± SE (4 h)						
0.0001	27 ± 6.18 <sup>Cc</sup>	29.15± 8.37 <sup>Cc</sup>	30.24±4.17 <sup>Cc</sup>	22.99±4.76 <sup>Cc</sup>	22.99±4.77 <sup>Cc</sup>	20.40±5.77 <sup>Cc</sup>	II
0.001	52.60± 5.20 <sup>Bb</sup>	39.78±5.47 <sup>Bb</sup>	52.60±5.20 <sup>Bb</sup>	55.16±5.94 <sup>Bb</sup>	40.04±4.13 <sup>Bb</sup>	50.06±5.19 <sup>Bb</sup>	III
0.01	90.89 ± 6.12 <sup>Aa</sup>	90.89±6.12 <sup>Aa</sup>	99.98±9.19 <sup>Aa</sup>	96.39±4.90 <sup>Aa</sup>	93.29±7.14 <sup>Aa</sup>	86.66±6.95 <sup>Aa</sup>	V

Values followed by the same small letters within a column and capital letters within a row are not significantly different ( $p < 0.05$ ) according to the LSD test. Lantana oil extracted from; UM1-KK-Upper Midland 1 Kakamega, LM-KI- Lower Midland-Kiboko, CL-MT- Coastal Lowland-Mtwapa, LH-NJ- Lower Highland-Njoro, UM3-EM- Upper Midland 3-Embu, UM2-KA- Upper Midland 2-Kandara.

different EO formulations and concentrations of *L. camara* leaf EO. The results revealed that there was no significant difference between the EO formulations ( $F = 1.68$ ;  $df = 5$ ;  $p = 0.1505$ ) and the interaction between the variables, dosage concentration and EO formulations interaction ( $F = 0.77$ ;  $df = 10$ ;  $p = 0.6539$ ) after 4 h of exposure. The EO of *L. camara* formulations showed significant repellent activity against the second instar larvae of *T. absoluta* at all concentrations ( $F = 156.28$ ;  $df = 2$ ;  $p < 0.0001$ ) within the 4 h of exposure. The repellency activity of *L. camara* EO was dose-dependent. The repellency increased when dosage levels increased. Complete repellency was observed when the highest dose level (0.01 µl/µl) was applied although there was a slight variation in the repellent effects within EO formulations. LM-KI EO showed the most repellency activity with 99.97% of repellency induced by 0.01µl/µl whereas the treatment of 0.0001 µl /µl induced only 30.24% of repellency of *T. absoluta* larvae, with an average rate of 60.93%. UM3-EM EO showed the lowest repellency activity within 4 h with 86.66% repellency induced by 0.01 µl/µl whereas the dose of 0.0001 µl/µl induced only 20.40% of repellency of *T. absoluta* larvae, with an average rate of 55.70%. Based on the mean repellency rate, EO formulations of all the *L. camara* leaves from diverse climatic zones showed repellency classes II, III and V with increasing dosage.

## DISCUSSION

Essential oils from plants produce a significant amount of valuable bioactive compounds. These compounds are mixtures of monoterpenes, sesquiterpenes, phenols, aldehydes, alcohols or other compounds (Olayemi, 2017). Compound accumulation and yield variations in EO composition are rather typical even within the same species. It depends on the genotype, plant organ, harvest, geographical region, season, plant nutritional status, climatic conditions-temperature, humidity, and light intensity (Moustafa et al., 2016; Ncube et al., 2012; Pereira et al., 2019; Swamy et al., 2017). The oils of *L. camara* showed considerable variability in the chemical composition, percent constituent and oil yield from the

same species growing in diverse ecological conditions of Kenya. Our results corroborate Bendera (2007) and Syombua (2015), who also found variability in the chemical profile of *L. camara* EOs harvested from Maseno-western Kenya and the eastern part (Kitui and Machakos), respectively. Similarly, reports from the different parts of the world also show remarkable differences in the chemical composition (El et al., 2014; Khalid, 2019; Moustafa et al., 2016; Murugesan et al., 2016; Nea et al., 2017; Omoregie et al., 2016; Swagatika and Smaranika, 2017).

This study established that the EO of the *L. camara* wild population in Kenya is rich in sesquiterpene and monoterpene compounds. They are occupied mainly by hydrocarbons and oxygenated sesquiterpene compounds with an average of 36.68 - 22.73% and 22.49 - 11.28%, respectively. The sesquiterpenes were discriminated by the dominant presence of β-caryophyllene, which agrees with previous studies, such as Dos et al. (2019). They found β-caryophyllene content at 13 - 8.9% in Brazil, while Khalid (2019) and Dougnon and Ito (2019) found it as 17.9 and 16.7 - 8.9% in Egypt and Benin, respectively. On the other hand, Sabinene (12.54 - 3.19%) dominated the monoterpenes, corroborating the findings of Nea et al. (2017) with up to 9.0% relative abundance. These results are consistent with our study; however, there are some differences in the percentages of detected compounds related to genetic and environmental factors (climate, seasons, geography and geology) variability. Several versatile standard components were present in all the EO analyzed in this work, including; bicylogermacrene, spathulenol, eucalyptol, (E)-nerolidol, and caryophyllene oxide, which are beneficial bioactive compounds. Based on the above fact, *L. camara*, cultivation widely in Kenya, stands a chance as a source for the isolation of various natural compounds and their bioactive properties tested for pest management.

The oil yields from *L. camara* leaves obtained in different regions significantly varied (Table 1). The populations around Mtwapa had the highest yield, while the lowest yield was observed in Kakamega. The populations of Mtwapa grow on the coastal lowland characterized by high temperature and a reasonable amount of rains. The environments there are sandy soils

and have vast day/night temperature difference. These factors may have resulted in high essential oil contents. However, in Kakamega, the prevailing conditions were dominated by low average temperatures and higher precipitation, providing a conducive growth environment. The differences in the chemical composition and yield of essential oils can be related to climatic and geographic factors such as temperature, ultraviolet radiation, atmospheric pollution, altitude, water and nutrient availability, and the developmental stage of the plant and genetic factors.

This paper shows the bioactivity studies of *L. camara* EO as a potential bioinsecticide to manage the tomato leaf miner, *T. absoluta*. While previous reports showed the potential of *L. camara* oil to be effective in the control of lepidopteran pests (Chau et al., 2019; Javier et al., 2017; Kasmara et al., 2018; Murugesan et al., 2016), our study provides the observed bioactivity of the EOs of *L. camara* from various climatic zones against the second instar larvae of the leaf miner, *T. absoluta*, together with their repellence activity; thus contributing to a thorough understanding of the potential use of EOs in the management of agricultural pests.

Notwithstanding, *L. camara* EOs have undergone bioassay tests as insecticides on several pests, but only a few studies have focused on the tomato borer. Furthermore, most bioassays reported focuses on trials lasting for less than 48 h, whereas the lasting effects of these compounds are lacking. To the best of our knowledge, no study on *L. camara* EO bioactivity against *T. absoluta*, together with their toxicity differences from oils sourced from the same plant but differing climatic zones, and their repellency activity has been recorded. However, previously, several researchers have tested the EOs from other plants against *T. absoluta* larvae for toxicity (Abdel-Baky and Al-Soqeer, 2017; Allam Tarik, 2015; Chegini et al., 2018; Javier et al., 2017; Khaoula, 2013; Moreno et al., 2012; Soares et al., 2019; Yarou et al., 2018) and their repellence activity (Allam Tarik, 2015). The EOs showed considerable toxicity and repellency activity on the *T. absoluta* larvae, comparable to the other plants EOs.

In our study, the insecticidal activity varied with the dosage concentration of the EO formulations. In the toxicity trial, the larval mortality was dose-dependent, and larval susceptibility increased with increasing dosage. Meanwhile, among the *L. camara* EO formulations, the larval mortality recorded did not significantly differ but differed substantially from the positive control (insecticide). Thus, the exposure concentration of the EO is proportional to the level of toxin and toxicity. Besides, the longer the exposure time, the higher the mortality rate recorded since longer time increases toxin accumulation. As shown by the LC<sub>50</sub> value of *L. camara*, EO formulations at 72 h of exposure showed a more substantial toxicity level.

The present study shows that leaf application of *L.*

*camara* EO is equally practical as a contact toxicant against the leaf miner, *T. absoluta*. Exposure of larvae to botanical compounds occurs through contact or systemic actions (Rwomushana et al., 2019). In particular, exposure to EO affected the larvae by weakening their development (Javier et al., 2017) and increased mortality. Larval mortality increased to over 80%, at the maximum dose concentration, showing that the EO of *L. camara* has larvicidal properties. For instance, in an experiment involving *T. absoluta* larvae, the neem extract at different concentrations resulted in 86.7 to 100% larval mortality (Elshiekh et al., 2014).

Besides, our study suggests that the *T. absoluta* second instar larval stage is relatively a critical stage to target the leaf miners. Eggs are less susceptible to insecticides (Elshiekh et al., 2014; Javier et al., 2017) because of the high numbers of enzymes that break down insecticides (Campbell et al., 2016). Our results were in line with those reported by Javier et al. (2017) and Kasmara et al. (2018), who found that *L. camara* EO caused over 50% larval mortality of *Spodopteralitura* (Lepidoptera). Other plants (eucalyptus, basil, castor bean, garlic, chinaberry, geranium, thyme, and onion) tested against *T. absoluta* larvae show insecticidal activity with different efficacies (Abd El-Ghany et al., 2016). The results also show that the *L. camara* EO has an outstanding repellency action on the second instar larvae of leaf miner, *T. absoluta*.

The volatile constituents such as  $\beta$ -caryophyllene, sabinene, bicylogermacrene, spathulenol, eucalyptol, (E)-nerolidol, thujene, caryophyllene oxide,  $\beta$ -copaene, davana ether, trans-chrysanthenol,  $\alpha$ -humulene epoxide, linalool, limonene, terpineol, terpene-4-ol,  $\alpha$ -pinene and camphor in the *L. camara* EO could be responsible for their observed insecticidal and repellent activity. Zandi-Sohani et al. (2012) also concluded that it might involve these components in the repellent and insecticidal activities against *C. maculatus*. A trial against *T. absoluta* larval stages using pure  $\alpha$ -pinene moderated toxicity effects was reported (Chegini et al., 2018). Limonene, 3-carene, terpinolene,  $\beta$ -myrcene and  $\gamma$ -terpinene have larvicidal activities to *Aedes aegypti* and *Aedes albopictus* larvae (Cheng et al., 2009). However, the role of a single compound in the EO is not definite, but the minor constituents act as synergists, enhancing the effectiveness of the EO (Akhtar and Isman, 2013; Hanem, 2012).

The EO formulations of *L. camara* were effective in controlling the target pest. Therefore, this could be a source of new bioinsecticide against *T. absoluta*. Further works may be necessary to assess their efficacy under realistic field conditions. However, there is a need to increase efficiency by developing methods that will allow for long-lasting effectiveness. Besides, there is no information on the potentially lethal and phytotoxic effects of *L. camara* EO on non-target organisms and target crop. Therefore, this is a new avenue for future research

and serves as a source of hypotheses for further research on *L. camara* essential oil as a potential bioinsecticide.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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