Bioactivity of Citrus jambhiri Peel and Eucalyptus globulus Leaf Essential Oils Against Chrysomya megacephala (Fabricius)

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Research Article

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Bioactivity of *Citrus jambhiri* Peel and *Eucalyptus globulus* Leaf Essential Oils Against *Chrysomya megacephala* (Fabricius)

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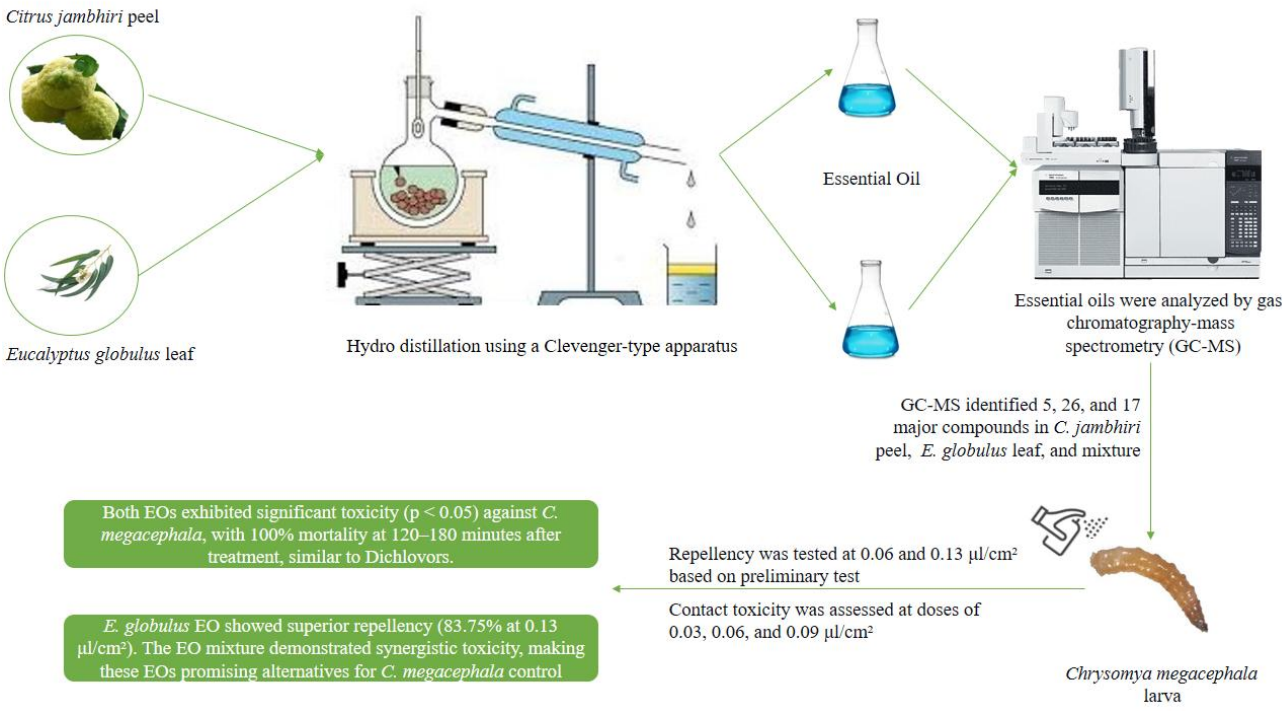
Abstract

Chrysomya megacephala, commonly known as Blow fly (latrine fly), thrives in human settlements, breeding in decaying organic matter and posing a public health risk by transmitting pathogens. This study examined the chemical composition and insecticidal efficacy of essential oils (EOs) obtained from *Citrus jambhiri* peel and *Eucalyptus globulus* leaf, individually and combined, against *C. megacephala*. EOs were extracted via hydro distillation using a Clevenger-type apparatus and analyzed by gas chromatography-mass spectrometry (GC-MS). Contact toxicity was assessed at doses of 0.03, 0.06, and 0.09 µL/cm², with dimethyl sulfoxide (DMSO) and Dichlorvos (DDVP) served as negative and positive controls, respectively. The repellency of the EOs was tested at 0.06 and 0.13 µL/cm², with acetone as a negative control. Treatments were replicated four times. Mortality was recorded at intervals up to 180 minutes after treatment (MAT), while repellency was evaluated up to 120 MAT. GC-MS identified five, twenty -six and seventeen major compounds in *C. jambhiri* peel, *E. globulus* leaf, and their mixture, respectively. *Citrus jambhiri* contained D-limonene (94.48%) and moslene (3.32%), while *E. globulus* had benzene (26.31%), spathulenol (15.69%), and α-pinene (6.86%) as major compounds. The EO mixture predominantly contained D-limonene (53.51%), γ-terpinene (22.63%), and myrcene (17.85%). Both EOs exhibited significant ($p < 0.05$) toxicity against *C. megacephala*, with 100% mortality at 120–180 MAT, similar to DDVP. *Eucalyptus globulus* EO showed superior repellency (83.75% at 0.13 µL/cm²). The EO mixture demonstrated superior toxicity compared with sole application, making these EOs promising alternatives for *C. megacephala* control.

Keywords: *Citrus jambhiri, Eucalyptus globulus, Chrysomya megacephala,* toxicity, essential oil



Graphical abstract

1. **Introduction**

Chrysomya megacephala (Diptera: Calliphoridae), commonly known as the oriental latrine fly or blow fly, is a synanthropic pest with significant public health implications globally [1]. Due to their feeding and reproductive behaviours, these blow flies serve as vectors for various microorganisms and pathogens affecting humans by contaminating food [2, 3]. They frequently infest and spoil products in meat, fish, and other animal-product industries, including slaughter houses and storage facilities, such as damage to stockfish [4, 5, 6]. Moreover, its larvae are associated with myiasis in both humans and animals, causing extensive damage and health complications [8, 9]. As necrophagous decomposers, they are among the first colonizers of animal carcasses, thus playing a role in decomposition [10]. Their close association with humans and ability to transmit pathogens make *C. megacephala* a major threat to both public health and animal welfare.

Effective control of *C. megacephala* populations is critical, and control methods include synthetic insecticides [11, 12], biological control agents [13], and Integrated Pest Management (IPM) approaches [14]. Although synthetic insecticides provide fast action, their persistent use

has led to pest resistance, environmental contamination, and adverse effects on non-target species [15]. Increasing dosages has led to resistance that exacerbates these issues, impacting soil fertility and bio-accumulation along the food chain [16]. Consequently, attention is shifting toward eco-friendly botanical alternatives, particularly essential oils (EOs). The key characteristics of EOs include their volatility, distinct aromatic profiles, and potential bioactivity against insects, which have shown promising results as biopesticides with reduced environmental impact [17,18,19]. Essential oils are complex blends of volatile compounds in different plant parts, including leaves, flowers, bark, roots, and fruits [20]. They have been isolated from species of different botanical families such as Asteraceae, Lamiaceae, Myrtaceae, Rutaceae, and Verbenaceae [21]. They are advantageous for insect control, as they can be effective in low concentrations without requiring direct contact with pests [22, 23].

Eucalyptus globulus, a member of the Myrtaceae family, is an aromatic medicinal plant with its EO being traditionally used for treating respiratory infections and fevers [24, 25, 26]. The EO of *E. globulus* was significant for its abundance of bioactive compounds, including phenolic acids, flavonoids, and tannins, which contribute to its antioxidant, antimicrobial, and insecticidal properties [27, 28, 29]. Similarly, *Citrus jambhiri* (Rutaceae) possess high amounts of bioactive compounds which can influence human health, as, e.g. vitamin C, carotenoids (β-carotene), flavonoids, etc. [30]. It possesses antioxidant, cardioprotective, anticarcinogenic, anti-allergic, antiplatelet, antiviral, antibacterial anticancer and antifungal activities [31, 32]. It has demonstrated fumigant repellency against storage pests [33, 34]. Studies have shown that the chemical profiles of botanical EOs can vary significantly based on factors like plant origin, environmental conditions, seasonal variation, effect of drying and harvest time, which may affect their efficacy [35, 36].

Despite prior studies on the insecticidal properties of *E. globulus* leaf and *C. jambhiri* peel EOs, research on the effectiveness of their single and combined application against *C. megacephala* is scarce. This study aims to characterize the chemical composition and assess the toxicity and repellency of EOs extracted from *E. globulus* leaves and *C. jambhiri* peel, individually and in combination, to provide an eco-friendly alternative for controlling *C. megacephala*.

2.0 Materials and Methods

This study was conducted in the Crop and Environmental Protection Laboratory and the Insect Rearing Shed of the Department of Crop and Environmental Protection, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria.

2.1 Insect Culture

The *C. megacephala* culture was established at the Insect Rearing Shed, Department of Crop and Environmental Protection, LAUTECH, Ogbomoso. Fresh poultry (layer) feces were collected from MAUD Poultry Farm, Stadium Area, Ogbomoso. Approximately 1 kg of the fecal sample was collected in a 15 x 60 cm rectangular plastic bowl with a netted opening for aeration. This culturing medium was set up in four replicates. Neonate larvae were monitored under ambient laboratory conditions (28±20°C temperature and 72±4% relative humidity) and maintained until pupation and adult emergence. The adults were authenticated by taxonomic verification at the Insect Collection Museum, Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria. Cultures were maintained by adding approximately 0.5 kg faecal samples from MAUD Farm on biweekly basis. Third-instar larvae were subsequently selected, rinsed with distilled water to remove adhered faecal matter, and prepared for bioassays.

2.2 Procurement of plant materials and extraction of essential oils

Fresh leaves of *Eucalyptus globulus* were collected from the University of Ilorin Campus, Ilorin, Kwara State, Nigeria (coordinates 8.4799^{0}N and 4.5418^{O}E) , and *Citrus jambhiri* peels were obtained from Oje Market, Ibadan, Oyo State, Nigeria (7.3881^{o}N and 3.9069^{o}E.). Plant samples were authenticated at the Botany Unit, Department of Pure and Applied Biology, LAUTECH, Ogbomoso. Essential oils were extracted by hydro distillation using the Clevenger apparatus. For each plant material, 500 g of fresh ground sample was combined with 2 liters of distilled water in a round-bottom flask, and hydro distillation was carried out for 3 hours [22]. The EOs were collected in airtight sample bottles and stored in a refrigerator until use for the bioassay and chromatographic analyistesting.

2.3 Chromatography procedure and identification of chemical constituents of the essential oils

The chemical constituents of the EOs were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS), following an established technique for EO profiling and compound identification [34]. In this procedure, a 1.0 µL sample of EO was injected into the GC-MS system, equipped with a high-resolution capillary column (Optima 5 MS) suited for separating volatile compounds [22]. The GC oven temperature was programmed to begin at 60°C, followed by a heating ramp of 10°C per minute up to 180°C, which was held for 2 minutes to allow separation of lower-boiling components. The temperature was then increased by 15°C per minute to 280°C, with a 4-minute hold at this final temperature to higher-boiling compounds [22]. The injector and interface temperatures were set at 250°C, ensuring efficient vaporization and transfer of the EO sample from the gas chromatography to the mass spectrometer.

The MS was operated in electron impact (EI) ionization mode at 70 eV, which is widely applied for organic compound analysis [38]. The ion source temperature was maintained at 200°C, with the detector covering a scan range of 400–700 m/z in full-scan mode, providing comprehensive spectra for compound identification [18]. Identification of the components was achieved by comparing the retention indices and mass spectra of each compound against those in the National Institute of Standards and Technology (NIST) mass spectral library and the literature [39]. Retention indices were calculated relative to an n-alkane homologous series, which provides reliable data on compound retention behavior under the same chromatography conditions [40]. Each compound with the highest similarity index (SI) to an authentic reference in the NIST database was accepted as the identified component for that spectrum. The relative concentrations of identified compounds were determined by peak area normalization based on the total ion chromatogram (TIC). Peak areas were expressed as a percentage of the total TIC, providing a quantifiable composition profile for each essential oil without correction factors [41, 42]. This normalization method facilitates the comparison of essential oil profiles and their potential biological activities.

2.4 Toxicity bioassay of essential oils of *Eucalyptus globulus* leaf and *Citrus jambhiri* peel against *Chrysomya megacephala* larvae

To assess the toxicity of essential oils from *Eucalyptus globulus* leaves, *Citrus jambhiri* peels, and their 1:1 (v/v) mixture against *C. megacephala* larvae, bioassays were conducted using Petri dishes (9 cm diameter) as exposure chambers. Three doses of EO (2, 4, and 6 µL) were prepared,

corresponding to surface concentrations of 0.03, 0.06, and 0.09 µL/cm², respectively. Each dose was dissolved separately in 0.2 mL of dimethyl sulfoxide (DMSO), which was applied to the bottom of the Petri dish using a micro-applicator to ensure even distribution. Control treatments included a negative control (0.2 mL of DMSO without essential oils) and a positive control Dichlorvos (DDVP). The positive control, Dichlorvos, was diluted in distilled water and applied at concentrations equivalent to those used for the essential oils (0.03, 0.06, and 0.09 µL/cm²). After the treatments, each Petri dish was allowed to sit for 2 minutes before introducing twenty (20) third-instar larvae in the dish. Each bioassay was replicated four times for statistical reliability. Mortality determination was done 10, 30, 60, 90, 120, 150, and 180 minutes after treatment (MAT). Larvae that exhibited no movement and failed to respond to a gentle probe with the blunt end of a pencil showed death.

2.5 Repellency bioassay of essential oils from *Citrus jambhiri* peel and *Eucalyptus globulus* leaf against *Chrysomya megacephala* larvae

To evaluate the repellency of EOs from *C. jambhiri* peel and *E. globulus* leaf against *C. megacephala*, an area preference test was conducted following the methodology established [33]. We utilized 9 cm-diameter Petri dishes, in which the EOs were formulated by dissolving the oils in acetone at concentrations of 0.06 µL/cm² and 0.13 µL/cm², with a total volume of 0.5 mL per solution. Half of a Whatman No. 1 filter paper was treated with acetone only, serving as a control in the experimental setup. The other half of the filter was filled with the EOs solutions. Both halves were allowed to dry for 10 minutes to ensure complete evaporation of the acetone before being placed at the bottom of each Petri dish. Twenty-third-instar larvae were kept at the center of the filter paper disc, and the Petri dishes were covered to prevent escape. Replication for each treatment was done four times to ensure statistical reliability. After 30, 60, 90, and 120 minutes, the treated and untreated larvae on the filter paper were estimated.

Percentage repellency (PR) was estimated using the formula:

$$PR = (Nc - N_T / Nc + N_T) \times 100$$

Where Nc = number of larvae on untreated disc

N_T =Number of larvae on treated disc.

2.6 Experimental design and data analysis

The experimental design was completely randomized design. Before analysis, data were subjected to arc-sine transformation. Thereafter, they were subjected to analysis of variance (ANOVA). Significant means were separated using Tukey's Honestly Significant Difference (HSD) at a 5% significant level. All data analyses were carried out using the SPSS Software 2020 Version.

3.0 Results

3.1 Chemical composition of *Citrus jambhiri* peel essential oil

The EO extracted from *C. jambhiri* peel was analyzed using GC-MS, revealing that monoterpenes were the primary chemical group. Five major compounds were identified. D-limonene was the most abundant chemical compound, accounting for 94.4% of the oil's total composition. Others are: moslene (3.32%), cyclohexene (1.1%), cyclofenchene (0.64%), and β-terpinene (0.46%) (Table 1).

3.2 Chemical composition of *Eucalyptus globulus* leaves essential oil

The EO from *E. globulus* leaf contained a more diverse profile, identifying twenty-six compounds. The chemical group consisted of monoterpene (39.63%), oxygenated monoterpene (13.09%), sesquiterpene (11.59%), Oxygenated Sesquiterpene (29.61), and others (6.02) making a total of 96.44%. The major compounds were o-cymene (26.31%), spathulenol (15.09%), α-pinene (6.83%), cyclopentanone (6.00%), viridefloral (5.61%); globulol (5.04%), with other minor compounds as presented in Table 2.

3.3 Chemical composition of a mixture of *Citrus jambhiri* peel and *Eucalyptus globulus* leaf essential oil (EO)

The chemical composition of the mixture of the EO from *C. jambhiri* peel and *E. globulus* leaf showed sixteen significant compounds. The chemical groups of the compounds consisted of monoterpene (97.18%), oxygenated monoterpene (1.63%), and sesquiterpene (0.15%), with a total of 98.96%. The major monoterpenes identified were D-limonene (53.51%), γ-terpinene (22.63%), and myrcene (17.84%). Other monoterpenes, including β-thujene (0.05%), β-phellandrene (1.04%), and β-pinene (0.99%), were present in lower quantities. Additionally,

oxygenated monoterpenes, represented solely by terpinen-4-ol (1.63%), and oxygenated sesquiterpenes, with a minor presence of globulol (0.09%) were also detected. Caryophyllene (0.15%) was the sesquiterpene identified in the mixed EO (Table 3).

Table 1: Chemical compounds identified in the essential oil of Citrus *jambhiri* peel

S/N	Retention time	Retention index	Compound name	% Composition	Formula	Chemical group	Mode of identification
1	5.119	729	Cyclofechene	0.64	$C_{10}H_{16}$	Monoterpene	GC-MS R1
2	5.579	964	Cyclohexene	1.10	$C_{10}H_{16}$	Monoterpene	GC-MS R1
3	5.633	993	-beta-terpinene	0.46	$C_{10}H_{16}$	Monoterpene	GC-MS R1
4	6.310	1018	D-Limonene	94.48	$C_{10}H_{16}$	Monoterpene	GC-MS R1
5	6.712	998	Moslene	3.32	$C_{10}H_{16}$	Monoterpene	GC-MS R1

Summary: Monoterpene – 99.98%

GCMS-RI: Gas Chromatography-Mass Spectrometry Retention Index

Table 2. Chemical Compound identified in the essential oil of *Eucalyptus globulus* leaf

S/N	Retention time	Retention index	Compound name	% Composition	Formula	Mode of identification
1.	5.017	902	3-thujene	2.13	$C_{10}H_{16}$	GC-MS R1
2.	5.108	729	α-pinene	6.83	$C_{10}H_{16}$	GC-MS R1
3.	6.183	2139	o-Cymene	**26.31**	$C_{10}H_{14}$	GC-MS R1
4.	8.236	1119	1-terpineol	2.77	$C_{10}H_{14}$	GC-MS R1
5.	8.411	1143	terpinol, alpha	1.23	$C_{10}H_{18}O$	GC-MS R1
6.	8.754	1064	2-ethyl-2-hexenal	0.99	$C_7H_{10}O_2$	GC-MS R1
7.	8.958	1463	2-methyl-propione acid	1.45	$C_9H_{14}O_3$	GC-MS R1
8.	9.133	1130	2-Isopropyl-5methyl	1.64	$C_0H_{16}O_2$	GC-MS R1
9	9.849	1745	6-(2,6-dimethylhapty	3.23	$C_{15}H_{26}O$	GC-MS R1
10	9.953	1745	cyclopantanone	6.00	$C_{15}H_{26}O_2$	GC-MS R1
11	10.929	1184	1-benzy(4-nmyl(2Z)	2.06	$C_{12}H_{20}O$	GC-MS R1
12	11.333	1419	IH-cycolprol(e) azuel	0.50	$C_{15}H_{24}$	GC-MS R1
13	11.673	1386	alloaromedandrene	5.84	$C_{15}H_{24}$	GC-MS R1
14	11.903	1386	aromandendrene	2.56	$C_{15}H_{24}$	GC-MS R1
15	12.283	1419	(+)-ledene	1.00	$C_{15}H_{24}$	GC-MS R1
16	12.973	1530	epiglobulol	0.57	$C_{15}H_{26}O$	GC-MS R1
17	13.033	1530	virideflorol	5.61	$C_{15}H_{24}$	GC-MS R1
18	13.122	1536	spathuleneol	15.69	$C_{15}H_{24}O$	GC-MS R1
19	13.180	1536	(-) spathulenol	1.14	$C_{15}H_{24}O$	GC-MS R1
20	13.232	1530	globulol	5.04	$C_{15}H_{26}O$	GC-MS R1

21	13.392	1598	2-(4a,8-dimethy (2,3,4)	0.75	$C_{15}H_{26}O$	GC-MS R1
22	13.442	1536	(-) spathulenol	1.21	$C_{15}H_{24}O$	GC-MS R1
23	13.700	1518	α-issbolene	0.85	$C_{15}H_{24}$	GC-MS R1
2	15.458	969	p-mentha-1,5-diene	1.59	$C_{10}H_{16}$	GC-MS R1

Summary: Monoterpene -39.63%, Oxygenated Monoterpene – 13.09%, Sesquiterpene – 11.59%
Oxygenated Sesquiterpene – 29.61, others – 6.02 Total – 96.44%

Table 3. Chemical compounds obtained from the mixture of essential oils of *Citrus jambhiri* peel and *Eucalyptus globulus* leaf

S/N	Retention time	Retention index	Compound Name	% Composition	Formula	Mode of Identification
1	5.03	873	β-thujene	0.05	$C_{10}H_{16}$	GC-MS RI
2	5.567	964	β-homolene	1.04	$C_{10}H_{16}$	GC-MS RI
3	5.808	943	β-pinene	0.99	$C_{10}H_{16}$	GC-MS RI
4	6.183	1042	myrcene	17.84	$C_{10}H_{14}$	GC-MS RI
5	6.335	1018	D-limonene	53.51	$C_{10}H_{16}$	GC-MS RI
6	6.55	976	β-ocimene	0.03	C_9H_{14}	GC-MS RI
7	6.692	998	γ-terpinene	22.63	$C_{10}H_{16}$	GC-MS RI
8	6.877	948	dita 3-carene	0.6	$C_{10}H_{16}$	GC-MS RI
9.	8.251	1137	terpinen-4-01	1.63	$C_{10}H_{18}O$	GC-MS RI
10	11.446	1494	caryophyllene	0.15	$C_{15}H_{24}$	GC-MS RI
11	11.607	1430	α-bergamotene	0.2	$C_{15}H_{24}$	GC-MS RI
12	11.7	1339	(+)-satrven	0.11	$C_{15}H_{24}$	GC-MS RI
13	11.984	1490	α-guaiene	0.2	$C_{15}H_{24}$	GC-MS RI
14	12.142	1515	germacrene	0.1	$C_{15}H_{24}$	GC-MS RI
15	12.298	1431	elixene	0.04	$C_{15}H_{24}$	GC-MS RI
16	13.284	1530	globulol	0.09	$C_{15}H_{26}O$	GC-MS RI

Summary: Monoterpene -97.18%, Oxygenated Monoterpene – 1.63%, Sesquiterpene – 0.15%, Total – 98.96%

Table 4: Contact toxicity of *Citrus jambhiri* peel essential oil against *Chrysomya megacephala* larvae

	Mean (%) mortality/Minutes After Treatment						
Treatment (µL/cm²)	**10**	**30**	**60**	**90**	**120**	**150**	**180**
0.03	2.50± 0.56c	36.25±1.86bc	53.75±0.97bc	73.75±1.22bc	88.75±0.91ab	83.75±1.06ab	87.50±0.86b
0.06	33.75±1.35b	50.00±1.31b	62.50±1.43b	80.00±1.28b	90.00±0.88a	98.75±0.39a	100.00±0.00a
0.09	36.25±1.28b	65.00±1.56ab	80.00±0.97ab	90.00±0.95a	93.75±0.88a	98.75±0.56a	100.00±0.00a
DMSO	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00a
Dichlorvos	100±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a

Values are means ± SE of four replicates. Means with the same alphabets within the column are not significantly different usingukey's HSD Test ($p \leq 0.05$)

3.4 Contact toxicity of *Citrus jambhiri* peel essential oil against *Chrysomya megacephala* larvae

The EO of *C., jambiri* was toxic to *C. megacephala* larvae, and the toxicity was dose-dependent. At the lowest concentration (0.03 µL/cm²), mortality increased gradually from 2.5% at 10 Minutes After Treatment (MAT) post-treatment to 88.75% at 120 MAT and progressed to 180 MAT, respectively. This indicates that this concentration induced moderate mortality over time. The medium concentration (0.06 µL/cm²) showed more potent initial effectiveness, with 33.75% mortality at 10 MAT and rose steadily, reaching 90% at 120 MAT and achieving 100% mortality at 180 MAT. At dose of 0.09 µL/cm², the EO had the most significant toxic impact. Beginning with 36.25% (mortality at 10 minutes, it rose to 80% by 60 minutes and reached 90% at 90 minutes achieving 100% 180 minutes, which was at par with Dichlorvos. The DMSO (negative control) showed 0% mortality across all time intervals (Table 4).

3.5 Contact toxicity of *Eucalyptus globulus* leaf essential oil (EO) against *Chrysomya megacephala* larvae

The contact toxicity of *E. globulus* leaf EO against *C. megacephala* larvae was also dose-dependent over the exposure period (Table 5). At 60 MAT, the EO concentrations of 0.09 µL/cm² induced significant larval mortality (85.00±1.50a) and 100% mortality from 90 MAT (100.00±0.00a) to 180 MAT as DDVP. Also, 0.06 µL/cm² dose evoked 100% mortality from 120 MAT (100.00±0.00). In contrast, 0.03 µL/cm² evoked 100% mortality at 150 MAT (97.50±0.56a) of *C. megacephala,* demonstrating toxicity levels comparable to those of Dichlorvos, highlighting the potential of *E. globulus* leaf EO as an effective larvicidal agent under different exposure (Table 5).

Table 5. Contact toxicity of essential oil of *Eucalyptus globulus* leaf against *Chrysomya megacephala* larvae

	Mean (%) mortality/Minutes After Treatment (MAT)						
Treatment (µL/cm^2)	10	30	60	90	120	150	180
0.03	17.50±0.63cd	28.75±0.73b	50.00±0.82b	68.75±0.77b	83.75±0.77b	97.50±0.56a	97.50±0.56a
0.06	27.50±0.56cd	31.25±0.80b	36.25±0.82b	50.00±0.94c	100.00±0.00a	100.00±0.00a	100.00±0.00a
0.09	56.25±1.27b	62.50±1.44ab	85.00±1.50a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a
DMSO	0.00±0.00d	0.00±0.00c	0.00±0.00c	0.00±0.00d	0.00±0.00c	0.00±0.00b	0.00±0.00b
Dichlorvos	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a

Values are means ± SE of four replicates. Means with the same alphabets within the column are not significantly different using Tukey's HSD Test ($p \leq 0.05$)

Table 6. Effect of C*itrus jambhiri* peel and *Eucalyptus globulus* leaf essential oil mixture against *Chrysomya megacephala* Larvae

Treatment (µL/cm²)	Mean (%) mortality/Minutes After Treatment (MAT)						
	10	30	60	90	120	150	180
0.03	40.00±0.79b	51.25±0.73b	63.75±0.39c	77.50±0.39b	100.00±0.00a	97.50±0.56a	97.50±0.56a
0.06	11.25±0.91c	40.00±0.96b	96.25±0.55a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a
0.09	85.00±10.80a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a
DMSO	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00b	0.00±0.00b
Dichlorvos	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a

Values are means ± SE of four replicates. Means with the same alphabets within the column are not significantly different using Tukey's HSD Test ($p \leq 0.05$)

3.6 Toxicity of *Citrus jambiri peel* and *Eucalyptus globulus* leaf essential oil mixture against *Chrysomya megacephala* larvae

The combined application of EOs from *C. jambhiri* peel and *E. globulus* leaf demonstrated more toxicity than the single EO application. At 10 MAT, a 0.09 µL/cm² concentration induced 100% mortality. Similarly, at 30 MAT, 0.06 µL/cm² produced results that were not significantly different from Dichlorvos (DDVP), which caused 100% mortality. At 120 MAT, 0.03 µL/cm² also achieved 100% mortality of *C. megacephala*. The mixture of the EOs applied at 0.09 µL/cm² elicited the highest larval mortality and effectively competed with DDVP at 10 MAT (Table 6).

3.7 Repellency of *Citrus jambhiri* peel and *Eucalyptus globulus* leaf essential oils against *Chrysomya megacephala* larvae

The result shows the toxicity of a sole application of *C. jambiri* and *E. globulus* against *C. megacephala*. All tested doses displayed repellent activity against *C. megacephala* at different time exposures. Application of the mixed EO at 6.6 µL/cm² and 0.13 µL/cm² exhibited a repellence of 78.75, 62.50, 85.00, 68.75%, and 68.75, 78.75, 62.50, 52.50% at 30, 60, 90, and 120 MAT, respectively. At 30 and 60 MAT, the 0.13 µL/cm² dose produced higher repellency than 0.06 µL/cm², though not significantly different. At 90 and 120 MAT, 0.06 µL/cm² had higher repellency when compared with 0.13 µL/cm² dose, still not significantly different (Figures 1 and 2).

The repellency of the mixture of *C. jambhiri* and *E. globulus* EO against *C. megacephala* larvae showed that at 6.6 µL/cm² and 0.13µL/cm² the mixed EO exhibited a repellency of 81.25, 80. 65.50, 52.50 and 100, 90, 91.25, and 91.25% at 30, 60, 90 and 120 MAT, respectively. The trend showed a decline in repellency with an increase in exposure time (Figure 3).

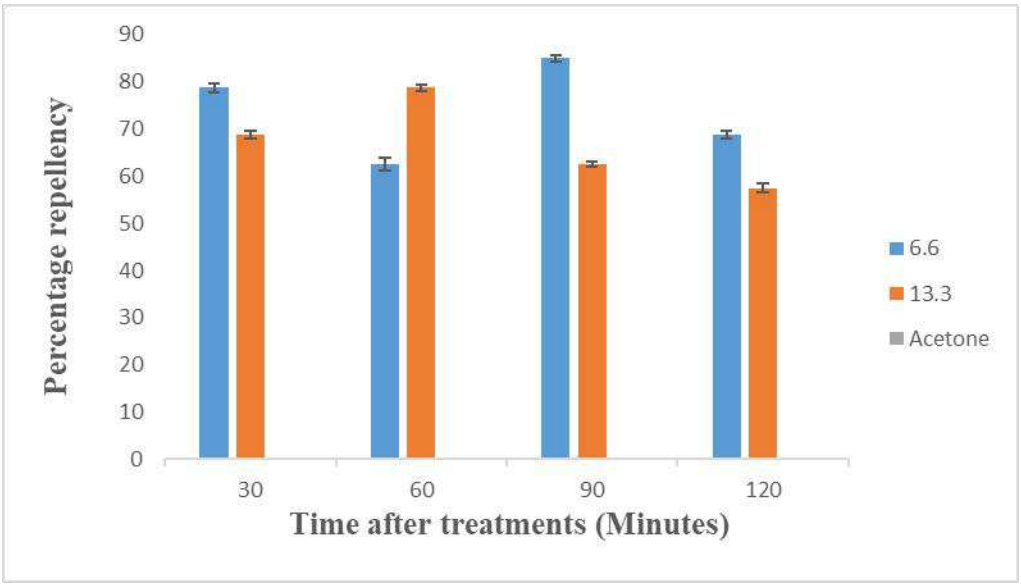


Figure 1. Repellency of *Citrus jambhiri* peel against *Chrysomya megacephala* larvae

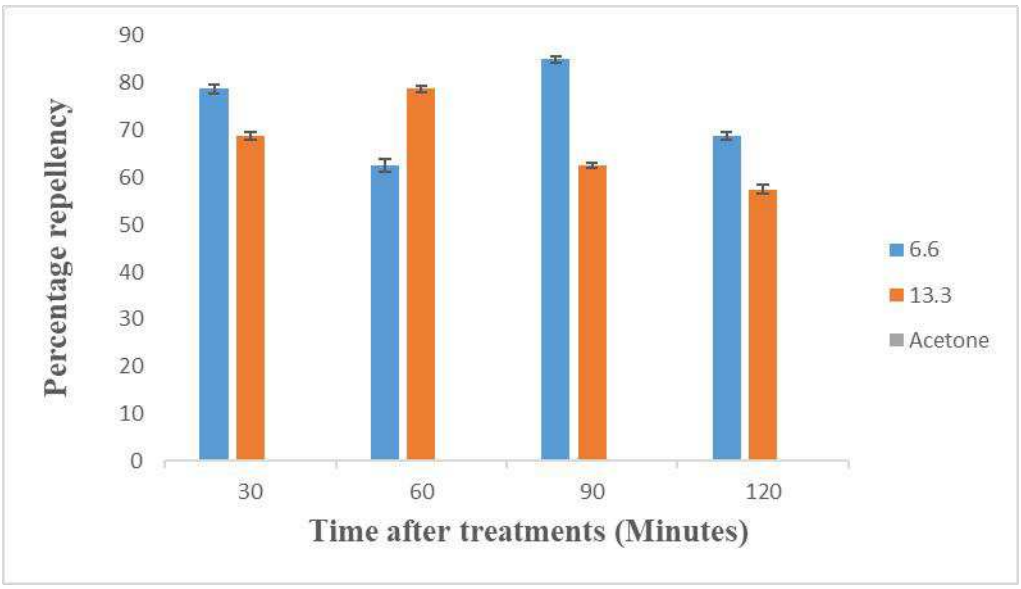


Figure 2: Repellency toxicity of *Eucalyptus globulus* leaf essential oil against *Chysomia megacephala* larvae

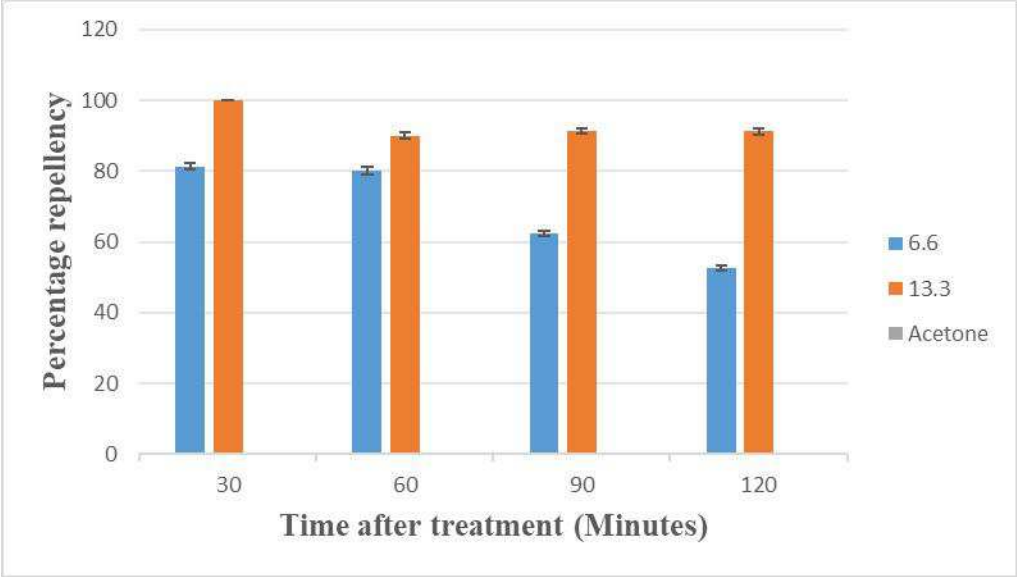


Figure 3: Repellency of *Citrus jambiri* peel and *Eucalyptus globulus* leaf essential oil mixture against *Chysomia megacephala* larvae

Discussion

In this study 5 compounds were observed from the *C. jambhiri* peel EO (D-limonene (94.4%), moslene (3.32%), cyclohexene (1.1%), cyclofenchene (0.64%), and β-terpinene (0.46%). The result is in contrast with [34] who reported 51 compounds in *C. jambhiri* fruit peel EO, with α-terpineol (8.034%) -terpineol (6.52 %), n-hexadecanoic acid (4.70 %), lemonol (7.67 %), citral (7.00 %), caryophyllene (4.58 %), citronellol (4.38 %), β-bisabolene (4.01 %), α-bergamotene (3.74 %), and Precocene I (3.33 %) as significant compounds. Also, another author [69] reported 25 compounds from the peel of *C. jambhiri* fruits by using two-dimensional GC-TOFMS analysis with cis-linalool oxide (19.85 %), terpinene-4-ol (7.37 %), trans-linalool oxide (furanoid) (14.86 %), and Limonene (4.64 %) were the main compounds of the *C. jambhiri* fruit peel oil. Seventy compounds were identified in the peel oil of *C. jambhiri* fruit, with β-bisabolene (4.52 %) and Limonene (60.81 %) as significant compounds.

Despite variations in the compounds obtained by different authors, *C. jambhiri* peel EO was dominated by D-limonene (94.48%) in this study, which aligns with findings of other authors [43, 44], who observed higher D-limonene (>610%) concentration in *C. jambhiri* peel EO. Similarly, previous authors [59] observed 92.48% D- limonene

in *C. jambhiri* being the highest compound. Other major compounds identified in this study have been reported in *C. jambhiri* obtained from around the globe [45].

Comparatively, *E. globulus* EO in this study contained 26 compounds. Monoterpene 39.63% was the highest, which is in agreement with the observation of [46] who reported 99.2% of monoterpenes predominantly o-cymene (26.3%). Also, it is in agreement with [47] who also identified 26 compounds in *E. globulus* leaf EO from Algeria. The *E. globulus* EO was also characterized by other principal components, such as spathulenol (15.69%) and alloaromadendrene (7.92%), aligning with the work of [48]. A similar study in northern Nigeria by [49] observed fewer compounds but with higher oxygenated monoterpene content, indicating geographical and environmental influences on EO composition. The variations in the chemical composition of the studied botanical EOs were attributed to chemotype, the studied plant part, extraction methods, variety, geographical location and harvest time [50, 51].

However, the combination of the EOs of *C. jambhiri* and *E. globulus* yielded 17 compounds, predominantly monoterpenes (97.18%), with D-limonene (53.31%) as the primary constituent, followed by gamma-terpinene (22.63%) and myrcene (17.84%). These findings support previous work by [52], which noted the predominance of monoterpenes in mixtures of *C. jambhiri* EOs. The main compounds identified in the EOs were D-limonene in *C. jambhiri* and o-cymene in *E. globulus*, both of which have established biological activity, including insecticidal and repellent properties [53, 54, 55].

The toxicity of *C. jambiri* EOs showed that the highest concentration was able to evoke 100% mortality of *C. megacephala* and compete with DDVP after 90 minutes and progressed with the exposure period. This result is in agreement with previous autors [34] who reported that the EO of *C. jambhiri* fruit rind was toxic against *Tribolium castaneum* adults, and the toxicity was dependent on the applied EO dose and progressed with exposure period throughout the experimental period. On the other hand, *E. globulous* EO at the same concentration evoked the highest mortality at 60 MAT, which agrees with earlier studies [56, 57] where *E. globulous* EO demonstrated toxicity to larvae and pupae of *Musa domestic*. Also, the insecticidal

activity of *E. globulous* against *Oxycarenus lavaterae* has been reported to be dependent on the applied EO concentration [53].

The mixture of both concentrations caused the highest mortality at the highest dose (0.09 µL/cm²) at 10 MAT, and the lowest concentration was able to evoke mortality at 150 MAT. This shows that the combination of the two oils evoked superior toxicity against *C. megacephala.* Mixtures of *C. jambhiri* and *E. globulus* EOs demonstrated higher toxicity through contact exposure, leading to effective mortality among *C. megacephala* larvae and corroborating the earlier finding on the synergistic effect of EO mixtures [58]. Furthermore, D-limonene, the dominant compound in *C. jambhiri*, has been widely reported for its insecticidal properties [33]. In this study, combination of both EOs produced better results than when either of them was used singly against *C. megacephala,* In another assay combination of Rough lemon and Ponderosa lemon produced better anti-inflammatory agents and showed substantial antimicrobial activity against all tested Gram-positive bacteria and yeasts [59].

Both EOs were able to repel *C. megacephala* larvae at different times. *Citrus jambhiri* and *E. globulus* EOs performed effectively against *C. megacephala* larvae but at different dosages. This study agrees with [60], who reported that behavioral responses of *T. castaneum* varied depending on the assay and the EO used, suggesting that repellency can fluctuate over time and under different conditions. The mixture of the EO demonstrated greater repellence at 0.13µL/cm² than at 6.6 µL/cm². As the dosage time increases, repellency reduces for both concentrations. This study agrees with [61] that repellency effectiveness diminishes as time progressed. At a 333 µg/cm² dose, *L. camara* and *S. terebinthifolia* exhibited repellency for up to 60 minutes against *An. Gambiae* and *S. terebinthifolia* were effective for 120 minutes [61]. The repellent property of the EOs establishes their potential for use to prevent the pest from infesting a treated substrate.

Previous studies have documented the bioactivity of *E. globulus* EO, which has been extensively studied for its insecticidal properties against various insect pests. The primary active constituents of E. globulus include 1,8-cineole, α-pinene, and d-limonene, which contribute to its efficacy [30]. On the other hand, *C. jambhiri* EO has been noted for its toxicity against *T. castaneum* [34]. The present study confirmed that

E. globulus against *C. megacephala* is an eco-friendly alternative natural insecticides [68].

Generally, EOs are known for their insecticidal activity due to the terpenoid compounds. Several EOs have been reported against insect pests such as *Rhodnius prolixus*, *Sitophilus zamias* and *Tribolium castaneum* [62, 63, 64, 65, 66] and other pests of stored agricultural products [67]. In the present study, the comparison of the bioactivity of the EOs with Dichlorvos, a synthetic insecticide, demonstrated earlier efficacy. Though, Dichlorvos achieved earlier and higher mortality, it poses risks of environmental toxicity and the potential for resistance development in target pests. Essential oils, by contrast, provide a safer alternative with promising insecticidal efficacy against *C. megacephala*. Since the combined EO exhibited comparative toxicity with Dichlorvos at prolonged duration of treatment, it shows potential as alternative to overdependence on the synthetic insecticide. The present study is the first study on *C. jambhiri* and *E. globulus against C. megacephala* in Nigeria. With the number of chemical compounds in the EOs, the tendency of *C. megacephala to* develop resistance against the EOs is low.The study recommends the use of *C. jambhiri* peel and *E. globulus* leaf EOs, as practical bio-insecticides against *C. megacephala*.

Abbreviations

C. *megacephala*	*Chrysomya megacephala*
C. jambhiri	*Citrus jambhiri*
T. castaneum	*Tribolium castaneum*
E. O	Essential Oil
E. globulus	*Eucalyptus globulus*
LSD	Least significant difference
ANOVA	Analysis of variance
SPSS	Statistical analysis system
HSD	honestly significant difference
GCMS	Gas chromatography–mass spectrometry
L. camara	*Latana camara*
LAUTECH	Ladoke Akintola University of Technology

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Authors' contributions

Study conception, experimental design and supervision were done by SA Babarinde, AF Odewole. Data collection and experimentation were carried out by IB Olatunji and AF Odewole. IB Olatunji, AF Odewole, and SA Babarinde handled data Analysis and manuscript drafting. All the authors have read and consented to publish the manuscript.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author (aodewole@lautech.edu.ng) on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable. The Blow fly used in this study was obtained from the poultry droppings obtained from a farm and was multiplied in accordance with applicable ethical, legal, and institutional guidelines. No specific permission was required for the use or collection of plant material in the study area. All experimental procedures complied with current research ethics standards and guidelines for scientific research involving biological materials. The plant materials were collected exclusively from the study site, the locations have been specified in the manuscript using geographic coordinates.

Consent for publication

All authors are agreeing for publication.

Competing interests

The authors declare that they have no conflicts of interest

References

1. Mandal, E., Amin, M. R., Rahman, H., and Akanda, A. M. Abundance and foraging behaviour of native insect pollinators and their effect on mustard (*Brassica juncea* L.). *Bangladesh Journal of Zoology*, 2018 *46*(2), 117-123.
2. Junqueira, A. C. M., Ratan, A., Acerbi, E., Drautz-Moses, D. I., Premkrishnan, B. N., Costea, P. I., ... and Schuster, S. C. The microbiomes of blowflies and houseflies are bacterial transmission reservoirs. *Scientific Reports*, 2017 *7*(1), 16324. https://doi.org/10.1038/s41598-017-16353-x
3. Stoffolano, J. G. Fly foregut and transmission of microbes. *Advances in insect physiology*, 2019 *57*, 27-95.
4. Aak, A., Birkemoe, T., and Mehl, R.. Blowfly (Diptera, Calliphoridae) damage stockfish in northern Norway: pest species, damage assessment and the potential of mass trapping. *Journal of Pest Science*, 2010 83, 329-337. https://doi.org/10.1007/s10340-010- 0302-9
5. Omalu, I., Egwim, E., Abdulraman, K., Ibrahim, S., Hassan, C., Eke, S., ... and Boyi, A. Free Radical Scavenging Activity and Protein Concentration and Profile of the Blowfly Maggot (*Lucilia robineau*) Excretion/Saliva Extract. *British Journal of Pharmaceutical Research* 2016, *9*(3), 1-6.
6. Debelts, H. . An Abattoir as a Multispecies Dwelling in Central Nigeria. *Science and Pollution Research* 2023 **27**(25), 31863-31871.
7. Sukontason, K. L., Narongchai, P., Sripakdee, D., Boonchu, N., Chaiwong, T., Ngern-Klun, R., ... and Sukontason, K. First report of human myiasis caused by *Chrysomya megacephala* and *Chrysomya rufifacies* (Diptera: Calliphoridae) in Thailand, and its implication in forensic entomology. *Journal of Medical Entomology*, 2005 *42*(4), 702-704.
8. Bernhardt, V., Finkelmeier, F., Verhoff, M. A., and Amendt, J. Myiasis in humans—a global case report evaluation and literature analysis. *Parasitology Research*, 2019 *118*, 389-397. https://doi.org/10.57187/s.3827
9. Caleffe, R. R. T., de Oliveira, S. R., Amat, E., Gomez-Piñerez, L. M., Daquila, B. V., Ruvulo-Takasusuki, M. C. C., and Conte, H. Larval internal morphology of the introduced blowfly *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae). *Zoologischer Anzeiger*, *310*, 2024 23-33. https://doi.org/10.1016/j.jcz.2024.03.003
10. Tan, S. H., Kurahashi, H., and Sawabe, K. Fly. In Medical Entomology *in Asia* 2024 (pp. 91-147). Singapore: Springer Nature Singapore. *https://doi.org/10.1038/s41598-024-61026-1*
11. Green, A. A. The control of blowflies infesting slaughterhouses is done through field observations of the habits of blowflies. *Annals of Applied Biology*, 1951 *38*(2), 475-494.
12. Gentz, M. C. A review of chemical control options for invasive social insects in island ecosystems. *Journal of Applied Entomology*,2009 *133*(4), 229–235.https://doi.org/10.1111/j.1439-0418.2008.01326.x
13. Caleffe, R. R. T., de Oliveira, S. R., Schoffen, R. P., de Oliveira Junior, V. A., and Conte, H. Biological control of Diptera Calliphoridae: A Review. *Journal of the Entomological Research Society*, 2019*21*(2), 144-155.
14. Cook, D. A historical review of management options used against the stable fly (Diptera: Muscidae). *Insects*, 2020 *11*(5), 313 **https://doi.org/10.3390/insects11050313**
15. Rizvi, S. A. H., Ling, S., and Zeng, X. *Seriphidium brevifolium* essential oil: A novel alternative to synthetic insecticides against the dengue vector *Aedes*

albopictus. *Environmental Science and Pollution Research*, 2020 *27*, 31863-31871. https://doi.org/10.1007/s11356-020-08924-3

16. Hossain, M. E., Shahrukh, S., and Hossain, S. A. Chemical fertilizers and pesticides: impacts on soil degradation, groundwater, and human health in Bangladesh. In Environmental degradation: challenges and strategies for mitigation 2022 (pp. 63-92). *Cham: Springer International Publishing.* https://doi.org/10.1007/978-3-030-95501-7_4
17. Elshafie, H. S., and Camele, I. An overview of the biological effects of some Mediterranean essential oils on human health. *BioMed Research International*, *2017*(1), 9268468. DOI: 10.1155/2017/9268468
18. Babarinde, G. O., Babarinde, S. A., Ojediran, T. K., Odewole, A. F., Odetunde, D. A., and Bamido, T. S. Chemical composition and toxicity of *Jatropha curcas* seed oil against *Sitophilus zeamais* Motschulsky as affected by pre-extraction treatment of seeds. *Biocatalysis and Agricultural Biotechnology*,2019 *21*, 101333. https://doi.org/10.1016/j.bcab.2019.101333
19. Maddheshiya, R., and Singh, K. P. Effect of *Lantana camara* essential oil on embryogenesis and postembryonic development of the Blowfly, *Chrysomya megacephala* (Diptera: Calliphoridae) following egg treatment. *Invertebrate Reproduction & Development*, 2022 *66*(1), 16-22. https://doi.org/10.1080/07924259.2021.2012576
20. Aqeel, U., Aftab, T., Khan, M.M.A. and Naeem, M. Regulation of essential oil in aromatic plants under changing environment. *Journal of Applied Research Medical Aromatical Plants2023 32*, .https://doi.org/10.1016/j.jarmap.2022.100441
21. Ramos da Silva, L. R., Ferreira, O. O., Cruz, J. N., de Jesus Pereira Franco, C., Oliveira dos Anjos, T., Cascaes, M. M., and Santana de Oliveira, M. Lamiaceae essential oils, Phytochemicals profile, antioxidant, and biological activities. *Evidence-Based Complementary and Alternative Medicine*, 2021(1), 6748052. https://doi.org/10.1155/2021/6748052
22. Babarinde, S. A., Pitan, O. O. R., Olatunde, G. O., and Ajala, M. O. Chemical composition of the essential oil of Nigeria-grown *Hoslundia opposita* Vahl (Lamiaceae) dried leaves and its bioactivity against cowpea seed bruchid. *Chemistry & Biodiversity*, 2017 *14*(6), e1600418 https://doi.org/10.1007/s11356-017-9589-x.
23. Odewole, A. F., Akinyemi, A. O., Babarinde, S. A., Olugbade, V. F., Ogunwale, O. R., Babatunde, T. G. and Adegboyega, P. O. Larvicidal activity of essential oil from *Citrus aurantifolia* peel against Fall Armyworm (*Spodoptera frugiperda* J.E. Smith). *Nigerian Journal of Horticultural Science 2023* 27(3) 85-9
24. Salari, M. H., Amine, G., Shirazi, M. H., Hafezi, R., and Mohammadypour, M. Antibacterial effects of *Eucalyptus globulus* leaf extract on pathogenic bacteria isolated from specimens of patients with respiratory tract disorders. *Clinical Microbiology and Infection*, 2006 *12*(2), 194-196.
25. Egwaikhide, P. A., Bulus, T., and Emua, S. A. Antimicrobial activities and phytochemical screening of extracts of the fever tree, *Eucalyptus globulus. Electronic Journal of Environmental, Agricultural & Food Chemistry*, 2010 *9*(5).
26. Lemos, I. C. S., de Araújo Delmondes, G., Dos Santos, A. D. F., Santos, E. S., de Oliveira, D. R., de Figueiredo, P. R. L., ... and Fernandes, G. P. . Ethnobiological survey of plants and animals used for treating acute respiratory infections in children of a traditional community in the municipality of Barbalha, Ceará, Brazil.

African J of Traditional, Complementary and Alternative Medicines, 2016 *13*(4), 166-175.https://doi.org/10.21010/ajtcam.v13i4.22

27. Boulekbache-Makhlouf, L., Slimani, S., and Madani, K. Total phenolic content, antioxidant and antibacterial activities of fruits of *Eucalyptus globulus* cultivated in Algeria. *Industrial Crops and Products*, 2013 *41*, 85-89. https://doi.org/10.1016/j.indcrop.2012.04.019
28. Dhakad, A. K., Pandey, V. V., Beg, S., Rawat, J. M., and Singh, A. A review of the biological, medicinal and toxicological significance of Eucalyptus leaf essential oil. *J of the Science of Food and Agriculture*, 2018 *98*(3), 833-848.
29. Sabo, V. A., and Knezevic, P. Antimicrobial activity of *Eucalyptus camaldulensis* Dehn. Plant extracts and essential oils: A review. *Industrial Crops and Products*, 2019 *132*, 413-429.https://doi.org/10.1016/j.indcrop.2019.02.052
30. Kumar, D., Ladaniya, M. S., and Gurjar, M. Underutilized Citrus sp. Pomelo (*Citrus grandis*) and Kachai lemon (*Citrus jambhiri*) are exhaled in phytochemicals and antioxidant potential. *J of food Science and Technology*, 2019 *56*(1), 217-223. https://doi.org/10.1007/s13197-018-3485-6
31. Anmol, R. J., Marium, S., Hiew, F. T., Han, W. C., Kwan, L. K., Wong, A. K. Y., ... and Ming, L. C. A review of the phytochemical and therapeutic potential of *Citrus grandis* (L.) Osbeck. *J of Evidence-based Integrative Medicine*, 2021 *26*, https://doi.org/10.1177/2515690X211043741
32. Diniso, T., Oriola, A. O., Adeyemi, J. O., Miya, G. M., Hosu, Y. S., Oyedeji, O. O., ... and Oyedeji, A. O. Citrus wastes: a valuable raw material for biological applications. *J of Applied Pharmaceutical Science*, 2024 *14*(8), 011-026.https://doi.org/10.7324/JAPS.2024.158781
33. Babarinde, S. A., Usman, L. A., Olaniran, O. A., Adebayo, T. A., Ojutiku, E. O., and Adeniyi, A. K. Toxicity and repellence of *Citrus jambhir*i Lush rind essential oil against maize weevil *(Sitophilus zeamais* Motschulsky 1855) (Coleoptera: Curculionidae). In *Proceedings of the 12th International Working Conference on Stored Product Protection, in Berlin, Germany on 7th–11th* 2018 (pp. 864-871).
34. Babarinde, S. A., Kemabonta, K. A., Olatunde, O. Z., Ojutiku, E. O., and Adeniyi, A. K. Composition and toxicity of rough lemon (*Citrus jambhiri* Lush.) rind essential oil against red flour beetle. *Acta Ecologica Sinica*, 2021 *41*(4), 325-331. https://doi.org/10.1016/j.chnaes.2020.07.001
35. Tanko, H., Carrier, D. J., Duan, L., and Clausen, E. Pre- and post-harvest processing of medicinal plants. *Plant Genetic Resources*, 2005 *3*(2), 304-313.
36. Babarinde, S. A., Bello, O. S., Olatunde, O. Z., Agboola, S. O. Arowolo, T. R. Volatile Compounds and Affecting Factors. In Singh, P. A. and Purewal, S. S. (Ed.) *Compendium of Clove* 2025 pp. 131-149. CRC Press. Taylor and Francis Group, United Kingdom.
37. Benbelaïd, F., Khadir, A., Rebai, R., Mebarki, S., Benziane, Y., Bendahou, M., and Muselli, A. Essential oils of *Thymus munbyanus* subsp. coloratus: chemical variability and evaluation of biological activities. *Journal of Essential Oil Bearing Plants*, 2024 *27*(2), 469-481. http:// doi.org/ **10.1080/0972060X.2024.2311117**
38. Yadegarinia, D., and Miri, R. Gas chromatography-mass spectrometry analysis of essential oils: A review. *Journal of Chromatography A*, 2020 *1627*, 462685. https://doi.org/10.1016/j.chroma.2020.462685
39. Gupta, A., Sinha, S. K., and Kumar, M. Advances in the extraction and analysis of essential oils: a review. *Natural Product Research*, 2022 *36*(14),

19661983.https://doi.org/10.57159/gadl.jcmm.2.3.23064.https://doi.org/10.1080/14786419.2020.1863693

40. NIST. 2011. NIST Chemistry WebBook, NIST Standard Reference Database Number 69. *National Institute of Standards and Technology.*
41. Salehi, B., Upadhyay, S., and Patra, J. K. Essential oils: Extraction, analysis, and their biological activities. *Natural Product Communications*,2021 *16*(3), 1-22. *https://doi.org/10.1177/1934578X211004993*
42. Yadegarinia, D., and Miri, R. Gas chromatography-mass spectrometry analysis of essential oils: A review. *Journal of Chromatography and sepatation techniquesA*,2020*1627*,462685. https://doi.org/10.1016/j.chroma.2020.462685.
43. Ismaeel, H. A., and Usman, L. A. A comprehensive review of the GC-MS analysis of essential oils and their biological activities. *Journal of Essential Oil Bearing Plants*, 2021 *24*(2), 118-135.
44. Priyadarshi, R., Naik, H., and Rathod, P. *The essential oil composition of Citrus jambhi* and its bioactivity. *Journal of Essential Oil Bearing Plants,* 2021 24(2), 150-157.
45. Kasali, A., and Olaniyan, O. Chemical composition of *Citrus jambhiri* leaf essential oil. *Journal of Essential Oil Research,* 2013 25(4), 334-337.
46. Mohammed, A. M. H., Ibrahim, M. A., Omran, A. A., Mohamed, E. M., and Elsheikh, S. E. Minerals content, essential oils composition and physicochemical properties of *Citrus jambhiri* Lush. (Rough Lemon) from the Sudan. *International Letters of Chemistry, Physics and Astronomy*, 2013 *9*, 25-30.
47. Čmiková, N., Galovičová, L., Schwarzová, M., Vukic, M. D., Vukovic, N. L., Kowalczewski, P.Ł., and Kačániová, M. Chemical composition and biological activities of *Eucalyptus globulus* essential oil. *Plants*, 2023 *12*(5), 1076. https; // doi.org/10.3390/plant12051076.
48. Harkat-Madouri, L., Asma, B., Madani, K., Said, Z. B. O. S., Rigou, P., Grenier, D., ... and Boulekbache-Makhlouf, L. Chemical composition, antibacterial and antioxidant activities of essential oil of *Eucalyptus globulus* from Algeria. *Industrial Crops and Products*, 2015 *78*, 148-153.
49. Luiz, F., Silva, A. J., and Pereira, L Chemical composition and insecticidal properties of *Eucalyptus globulus leaf essential oil*. *Industrial Crops and Products*, 2016 43, 50-58. https://doi.org/10.1016/j.indcrop.2016.07.017
50. Olayinka, A., Aliyu, R., and Ibrahim, S. Chemical composition and insecticidal activity of *Eucalyptus globulus essential oil from Nigeria*. *African J of Biotechnology*, 2012 11(17), 415-419.
51. She, Y. . Effects of environmental factors on essential oil composition. *Journal of Essential Oil Research,* 2020 32(5), 326-334.DOI: 10.1007/978-981-99-4370-8_10
52. Hadaib, F. Influence of environmental factors on the chemical composition of essential oils. *Phytochemistry,* 2002 59(3), 213-220.
53. Sousa, P., Moreira, R., and Rodrigues, F. Chemical composition and insecticidal properties of *Citrus jambhiri essential oil*. *Phytochemistry*, 2004 38(1), 67–72.
54. Čmiková, N., Galovičová, L., Schwarzová, M., Vukic, M. D., Vukovic, N. L., Kowalczewski, P.Ł., and Kačániová, M. Chemical composition and biological activities of *Eucalyptus globulus* essential oil. *Plants*, 2023 *12*(5), 1076. https; // doi.org/10.3390/plant12051076.

55. Sharma, P., Mehta, S., and Rana, V. A review on the use of essential oils for pest control. *Environmental Science and Pollution Research*, 2022 29(4), 4123-4135. https://doi.org/10.1007/s11356-021-15710-3
56. Pang, X., Lin, Y., and Huang, Z. Biological activities of D-limonene in pest control. *J of Agricultural and Food Chemistry,* 2022 69(20), 5617-5630. https://doi.org/10.1021/acs.jafc.1c01468
57. Kumar, P., Mishra, S., Malik, A., & Satya, S. Compositional analysis and insecticidal activity of *Eucalyptus globulus* (Family: Myrtaceae) essential oil against housefly (*Musca J of Medicinal Plants Research, domestic*). *Acta Tropica*, 2012 *122*(2), 212-218. https://doi.org/10.1016/j.actatropica.2012.05.005
58. Tak, J., Kumari, D., and Sharma, S. Synergistic effects of essential oils on insect mortality. *J of Entomological Science*, 2017 50(3), 300–309.
59. Hamdan, D., El-Readi, M. Z., Nibret, E., Sporer, F., Farrag, N., El-Shazly, A., and Wink, M. Chemical composition of the essential oils of two citrus species and their biological activities. *Die Pharmazie-An International J of Pharmaceutical Sciences*, 2010 *65*(2), 141-147.
60. Athanasiou, C. G., Phillips, T. W., and Aikins, M. J. Challenges in assessing repellency via the behavioural response of the global pest *Tribolium castaneum* to protect stored grains. *Insects, 2023 15*(8), 626. **https://doi.org/10.3390/insects15080626**
61. Nerio, L. S., Olivero-Verbel, J., and Stashenko, E. Chemical composition, repellent, and oviposition deterrent potential of wild plant essential oils against three mosquito species. *Molecules,* 2023 *29*(11), 2657. https://doi.org/10.3390/molecules29112657
62. Ko, K., Jang, J., and Kim, S. Repellent and insecticidal effects of essential oils on stored product pests. *Journal of Pest Science,* 2010 83(3), 247-254.
63. Zapata, N. and Smagghe, G.. Toxicity of essential oils on *Tribolium castaneum*. *J of Pest Science,* 2010 83(3), 247-254.
64. Suthisut, D., Fields, P., and Huang, W. Essential oils as potential insect repellents against stored grain pests. *Pest Management Science,* 2011 67(5), 586–592.
65. Sfara, V., Zerba, E. N., and Alzogaray, R. A. . Fumigant insecticidal activity and repellent effect of five essential oils and seven monoterpenes on first-instar nymphs of *Rhodnius prolixus*. *J of Medical Entomology*, 2014 *46*(3), 511-515.https://doi.org/10.1603/033.046.0315
66. Guru-Pirasanna-Pandi, G., Kumar, V., and Yadav, D. Essential oils as insect repellents against *Sitophilus oryzae*. *Journal of Entomology*, 15(1), 12-19.
67. Rajapake, C., and Ratnaseka, H. 2008. Repellent properties of essential oils against cowpea pests. *Tropical Agricultural Research,* 2018 20(4), 222-229.
68. Ainane, A.; Abdoul-Latif, F.M.; Abdoul-Latif, T.M. and Ainane, T. Evaluation of Biological activities of two essential oils as Safe environmental bioinsecticides:Case of *Eucalyptus globulus* and *Rosmarinus officinalis*. *Series*, 2020 *29*, 544-556
69. Ogunjinmi O. E, Olawore, N.O . Maharaj V.J Chemical examination of essential oil from stem, roots and fruit peels of Nigerian *Citrus jambhiri* J. Essential Oil Bear. Plants, 22 (2019), pp. 1614–1621.