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Insecticidal potential of essential oils from *Cupressus lusitanica* growing in ecological zones of Rwanda against adult housefly, *Musca domestica* L

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Abstract

The housefly, *Musca domestica* Linnaeus is a household pest mostly controlled by use of synthetic insecticides that often affect the environment and promote insecticide resistance. Plants of Cupressaceae family are often used locally in Rwanda and Uganda as an eco-friendly way to repel houseflies. In spite of this high utility of the plant, scientific proof of its efficacy is still limited. This study aimed at assessing the insecticidal activity of essential oils (EOs) from *Cupressus lusitanica* Mill. (Cupressaceae) growing in three agro-ecological zones (AEZs) of Rwanda; highland (Burera), midland (Huye) and lowland (Kayonza) against adult houseflies. EOs were extracted from fresh leaves using steam distillation method. Male and female adult houseflies were exposed to individual test solutions of 90, 70, 50 and 30% v/v (DDVP or EOs/Acetone) for 6, 12 or 24 h. The levels of repellant activity, contact and fumigant toxicity were then recorded. Acetone and DDVP insecticide served as negative and positive test controls, respectively. DDVP showed higher toxicity and repellant activity compared to tested EOs, whereas Acetone repelled less than 14% of exposed houseflies in 24 h. The EOs from leaves collected in lowlands had higher fumigant and contact toxicity, followed by that from midlands, while least toxic effects were observed for EOs from highlands. The higher repellant activity was exhibited by EOs from midlands, followed by oil from lowlands. The observed promising activity among investigated AEZs implies that the EOs of *C. lusitanica* could be used as alternative to synthetic insecticides for control of the housefly.

Keywords Cupressus lusitanica · Essential oils · Contact toxicity · Fumigant toxicity · Repellent activity · Agro-ecological zones

Introduction

The house fly, *Musca domestica* L. (common fly) is a worldwide pest challenging human health and his well-being. It lives in the areas where sanitation and hygiene are inadequate or have been compromised (Suresh et al. 2018). Housefly is believed to have originated from the steppes of

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central Asia in the Middle East and now inhabits all parts of the world as it adapts to different regions and climatic conditions (Khamesipour et al. 2018). Due to their adaptation, fecundity, ability to multiply rapidly and their arthropodal characteristics favoring its competitions with human population for scarce food and other ecological sources, houseflies are among the most successful insect pests that are ravaging the world (Ayo et al. 2019; Baana et al. 2018). Moreover, they are potential vectors of many pathogens leading to different infectious diseases including amoebic dysentery, shigellosis, salmonellosis, cholera, ascariasis, necatoriasis, enterobiasis and taeniasis (Soonwera and Sinthusiri 2014; Abbas et al. 2013).

The challenges caused by houseflies have made human beings to set up different control strategies aiming at the improvement of sanitation and hygienic conditions. These strategies include proper disposal of biodegradable wastes, removal of breeding sites and sources that may attract them leading to their rapid multiplication (Abbas et al. 2013). Even though these physical methods are easy to implement and safe, they are not very effective at combating a high density of houseflies (Wang-Na et al. 2019; Malik et al. 2007). Therefore, introduction and use of synthetic insecticides such as pyrethroids, organophosphates and carbamates became an inescapable way to control houseflies. However, the excessive and long-term use of synthetic insecticides has been linked to a variety of detrimental effects, including toxicity to non-target organisms, bioaccumulation, and increased persistence in the environment (Gangemi et al. 2016; Jairoce et al. 2016; Gill and Garg 2014; Aktar et al. 2009). Besides, houseflies have been reported to develop resistance leading to the failure of synthetic insecticides (Kole et al. 2019; Lushchak et al. 2018).

Botanical pesticides have been considered as safer alternatives to synthetic insecticides due to their higher rate of pests control as synthetic ones, extreme biodegradability and lower toxicity to mammals (Kamel et al. 2019; Gaire et al. 2017; Sola et al. 2014). Therefore, scientific activities aiming at discovering new efficient and environmentally friendly pesticides from plant origin products are of interest. The extracts and essential oils from different plants in Cupressaceae family have shown potential insecticidal activity against various household pests, including weevils (Bett et al. 2017; Yang et al. 2020), *Aedes albopictus* mosquitos (Giatropoulos et al. 2013) and houseflies (Baana et al. 2018; Elbermawy et al. 2011; Giatropoulos et al. 2013; Pavela 2008).

Moreover, different *Cupressus* species are locally used in different parts of Rwanda and Uganda to repel houseflies from people's settlements like houses, latrines and shops. The modes of application used include burning dried or fresh leaves and stem bark to generate smoke. Fresh leaves can also be placed in areas where houseflies are numerous or hang the branchlets on the roofs and walls of latrines and houses (Baana et al. 2018). This local practice implies the potential of *Cupressus* species as an alternative to synthetic insecticides.

Nonetheless, bioactivity of these plant species may vary from region to region due to the plant's habitat and its ecological and climatic features that are known to affect the chemical profile of plant's extract (Kabtni et al. 2020; Karami et al. 2020; Liambila et al. 2021). The management of their secondary metabolites including essential oils, and variations in biosynthesis have been suggested as mechanisms by which plants can withstand and adapt to environmental challenges (Barra 2009; Guedri et al. 2022; Moustafa et al. 2016). Therefore, it is worth to investigate the chemical profiles and bioactivities of essential oils from *C. lusitanica* M. in different ecological habitats. The aim of this study was to evaluate the insecticidal activity of essential oils from the leaves of *C. lusitanica* collected from three agro-ecological zones (AEZs) in Rwanda against adult housefly, *M. domestica*.

Materials, chemicals and reagents

Chemicals used in this study include commercial insecticide, Dichlorvos (Dimethyl 2, 2-dichlorovinyl phosphate, DDVP 77%) sourced from Loba Chemie PVT Ltd, Mumbai, India and Acetone solvent (analytical grade) purchased from Millipore Sigma, St. Louis, USA). Materials like petri dishes with lids (90 mm Diameter x 15 mm Deep), filter papers (Whatman-110 mm No.1), amber glass vials (4.0 mL), plastic jars, insect rearing cages ($50 \times 34 \times 37$ cm) and bioassay cages ($22 \times 15 \times 17$ cm) and equipment like digital hygrometer (HTC-2 Model, 10- 99% RH, accuracy: \pm 10% RH, \pm 1 °C, manufactured by Narayann Scientific Instrument Co. Ltd, New Delhi, India) and micropipette (0–20 µL), were used in this study, while food materials include milk powder, sugar, chicken eggs and wheat flour were locally sourced and provided to houseflies.

Plant material collection, essential oil extraction and analysis

Fresh leaves of *C. lusitanica* were collected in March and April 2021 from wild plant in three agro- ecological zones of Rwanda (Fig. 1). From each zone, three sampling locations were selected and their geographic coordinates are presented in Table 1.

Samples were then taken to the University of Rwanda, College of Science and Technology in the laboratory of Chemistry for identification of the plant species by a botanist from Department of Biology of the same University. The voucher specimen (No. 14427/001) were compared to those deposited under the name "Colete Nuyt 141", and deposited in the National herbarium at the University of Rwanda. After washing and removing foreign matter, fresh leaves were refrigerated at 4 °C for almost 15 hours, then subjected to steam distillation for essential oil extraction on the next day. Chemical analysis of essential oils was done in our previous research study using Fourier Transform Infrared Spectroscopy (FT-IR) and Gas Chromatography-Mass Spectrometry (GC-MS) and the detailed results were reported (Nteziyaremye et al. 2021). Some of the major compounds discovered and their chemical structures are presented in Table 2, and Fig. 2, respectively.

Housefly collection and maintenance

The starter colony of adult houseflies, *M. domestica* were collected from a slaughterhouse at Kesses market of Moi University, Uasin Gishu County, Kenya using a sweep net and plastic jars (3.5 L), then transported to the Biological

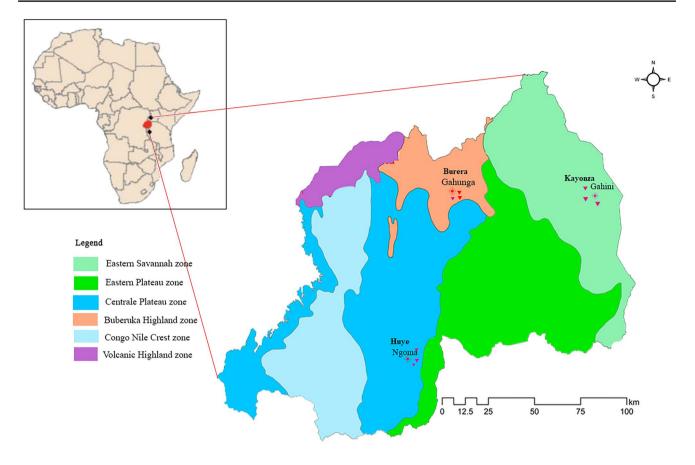


Fig. 1 Map showing agro-ecological zones of Rwanda with areas of the study (Mukuralinda et al. 2016)

Sciences Laboratory of Moi University for identification and rearing. The collected adult houseflies were reared in cages $(50 \times 34 \times 37 \text{ cm})$ and provided with different foodstuffs according to the methods described by Khater and Geden (2019) and Chintalchere et al. (2013).

A mixture of milk powder and granulated sugar at 1:1 ratio, bread soaked in the fresh milk, wheat flour and boiled eggs were provided for adults and replaced every two days, while tap water was given daily. Both, foodstuffs and water were provided to houseflies using plastic petri dishes (90 mm diameter x 15 mm deep). Cow dung placed in transparent plastic box (20×15 cm) served as breeding media and larval

Table 1 Location of sampling points of fresh leaves of Cupressus lusitanica

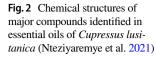
Region/AEZs	Sampling sites	Latitude (S)	Longitude (E)
Highlands	Burera	1°29'13.8" 1°27'10.2" 1°29'19.9"	29°40'44.7" 29°41'53.7" 29°39'44.3"
Midlands	Huye	2°35'30.3" 2°35'30.3" 2°37'11.3"	29°43'53.6" 29°43'59.3" 29°44'30.4"
Lowlands	Kayonza	1°51'29.3" 1°51'26.2" 1°51'26.6"	30°29'23.7" 30°29'22.8" 30°29'18.8"

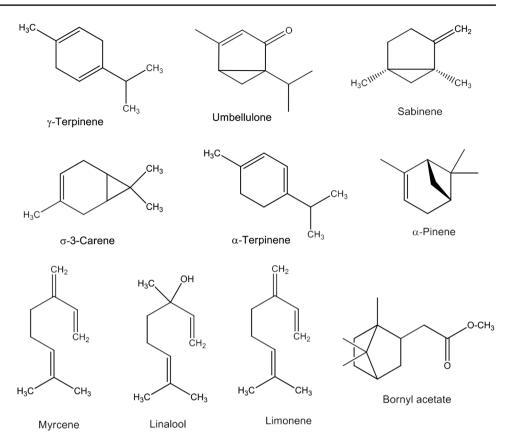
development substrate, while the pupae were kept in separate jars for adult emergence. Rearing and experimental conditions were maintained at a relative humidity (RH) of $65 \pm 5\%$ and temperature of 20 ± 2 °C, and the adult houseflies were continuously available for bioassay experiments.

 Table 2
 Major compounds identified in essential oil of Cupressus lusitanica and their abundances

Compounds	Abundance (%) as per ecological zone				
	Highlands (Burera)	Midlands (Huye)	Lowlands (Kayonza)		
α-Pinene	10.23	8.21	5.24		
Sabinene	20.84	10.54	4.05		
Myrcene	19.63	1.31	7.20		
Umbellulone	3.23	24.21	18.16		
δ-3-Carene	10.13	16.76	3.13		
γ-Terpinene	3.14	0.65	18.77		
Linalool	6.83	2.10	8.71		
Limonene	1.27	2.08	5.53		
Bornyl acetate	tr	-	9.72		
α-Terpinene	6.72	5.84	2.88		

tr trace < 0.10%, (-) not detected (Nteziyaremye et al. 2021)





Test solution preparation

Freshly prepared solutions of different concentrations (90, 70, 50 and 30% v/v) of test solutions (DDVP insecticide and Essential oils) were prepared by dilution with Acetone solvent (DDVP or EOs/Acetone). Solutions were prepared in amber vials covered with Aluminium foil, then refrigerated at 4 °C right away after preparation in Biological Sciences Laboratory of Moi University, Kenya until when used at the same day.

Contact toxicity bioassay

Contact toxicity was evaluated following the method described by Suwannayod et al. (2019) and Tian (2017) with a slight modification on experimental conditions of temperature $(25 \pm 3 \text{ °C})$ and relative humidity (50–70%), exposure time and method of anaesthetizing houseflies. A batch of 30 males and females, adult houseflies of 3–5 days old, were put in plastic jars (3.5 L) covered with a mesh and then anaesthetized by placing the jar in the fridge at 7–8 °C for 3 to 5 min. Anaesthetized houseflies were removed from fridge and put on white paper. One microliter (1.0 µL) from each test solution and controls was applied to the pronotum of each anesthetized housefly using micro-pipette (0–20 µL). Treated houseflies were transferred to bioassay cages ($22 \times 15 \times 17$ cm) (Fig. 3) and provided with sugar-milk

solution (10%). Adult houseflies' mortality was recorded after 6, 12 and 24 h of exposure to test solutions.

The housefly was defined as dead when it did not exhibit any movement after being prodded with a small brush (Paramasivam and Selvi 2017). Mortality of 5 to 20% in the negative control assay was corrected using Abbott's formula (Abbott 1925) (Eq. 1), while above 20% of mortality was rejected and the experiment repeated. Three replicates per experiment were done.

Corrected Mortality (%) =
$$\frac{X - Y}{100 - Y} \times 100$$
 (1)

with *Y*, the mortality (%) from negative control test;

and *X*, the observed mortality (%) from the essential oils tests or positive control (DDVP) test.

The lethal doses, LD_{50} and LD_{90} (the concentrations of essential oils or DDVP that kill 50% and 90% of the exposed adult houseflies, respectively) were obtained via Probit analysis of dose-mortality relationship.

Fumigant toxicity bioassay

Fumigant toxicity was evaluated following the method described by El-Sherbini and Osman (2014) and by Bande-borujeni et al. (2018). Thirty (30) male and female adult houseflies were placed in a 5 L plastic jar covered with mesh to facilitate ventilation (Fig. 4). The filter papers cut in the same diameter with

Fig. 3 Bioassay cages for essential oils (A, B and C) and controls (D and E) test experiments (Cage A, B and C were treated with EOs from HLZ, MLZ and LLZ, while D and E was for acetone and DDVP, respectively)



jar caps were separately impregnated with $100.0 \,\mu$ L of EOs and DDVP (90, 70, 50 and 30% v/v) and acetone (negative control). Each treated filter paper was attached to the inner surface of jar cap and protected with a piece of mesh in the way that prevented its direct contact with houseflies. Mortality of housefly was recorded in 6, 12 and 24 h, and three replications per experiment were done.

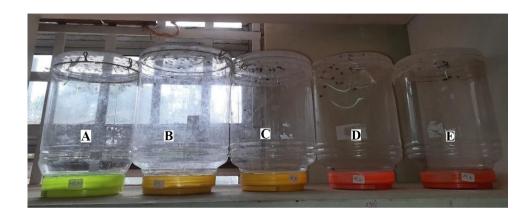
Mortality percentages were corrected using Abbott's formula in Eq. (1) (Abbott 1925), then converted into Probit values for calculation of lethal doses required to kill 50% (LD50) and 90% (LD90) of housefly population (Lopes et al. 2019).

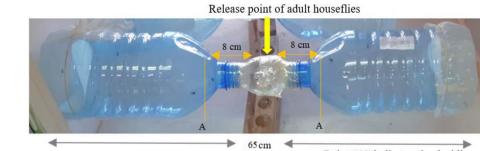
Repellency bioassay

Repellent activity of *C. lusitanica* essential oil against houseflies was evaluated following the method described by Chauhan et al. (2017) with modifications. The experimental setup consisted of two same size chambers made of transparent jars of 3.5 L with interconnecting passage where the houseflies were introduced (Fig. 5).

The filter papers (Whatman-110 mm thickness) impregnated with 50 µL of each EOs concentrations (90, 70, 50 and 30% v/v) were separately placed inside the end of one chamber, while the filter paper impregnated with 50 µL of acetone (Negative control) was placed inside the end of the opposite chamber. Similar procedure as EOs treatment assays was set for Dichlorvos insecticide which served as positive control. Thirty male and female adult houseflies (3-5 days old) were knocked down (by placing the jar in fridge at 7-8°C) and then introduced at release point (in the half-way of the two chambers) (Fig. 5) to allow the movement of their choice between two chambers. Housefly's movement was monitored and recorded in 6, 12 and 24 h of exposure by counting number of houseflies reached deciding Point A (Fig. 5) from the release point towards either test solution or acetone treated chamber. Number of houseflies moved toward the

Fig. 4 Bioassay jars for essential oils (**A**, **B** and **C**) and controls (**D** and **E**) test experiments (Jars **A**, **B** and **C** were treated with EOs of *C*. *lusitanica* from HLZ, MLZ and LLZ, while **D** and **E** was for Acetone and DDVP, respectively)





Point "A" indicates the deciding points

chamber treated with test solution (Nt) and negative control (Nc) were expressed in percentages (Eqs. 2 and 3).

Fig. 5 Laboratory scale set up

for housefly repellency test, with deciding points A

$$N_{t} = \frac{\text{No. of HFs moved toward EOs treated chamber}}{\text{No. of total HFs in assay}} \times 100$$
(2)

$$N_{c} = \frac{\text{No. of HFs moved toward Acetone treated chamber}}{\text{No. of total HFs in assay}} \times 100$$
(3)

All experiments were conducted in triplicates, and repellency percentages (PR) were calculated using Eq. (4).

Repellency percentage (PR) =
$$\frac{N_c - N_t}{N_c + N_t} \times 100$$
 (4)

with N_t and N_c , the percent of houseflies moved toward the test solution and acetone treated chambers, respectively.

Repellency data of 5 to 20% in negative control were corrected using modified Abbott's formula in Eq. (5) (Abbott 1925) while the repellence results higher than 20% in negative control were rejected prior to repeating experiment (Paramasivam and Selvi 2017).

Corrected repellency percentage =
$$\frac{N_t - N_c}{100 - N_c} \times 100$$
 (5)

Statistical analysis

The results were expressed as mean values \pm standard error of the three replicates. Significant differences between mean values were established through Tukey's honest significant difference (HSD) test (one-way ANOVA).

Data obtained from dose-response bioassays were subjected to Probit analysis (Finney 1971) to estimate LD_{50} and LD_{90} values for fumigant and contact toxicity bioassays, in 6, 12, and 24 h post-treatment. Mortality and repellency data between 5% and 20% in control assays were relatively corrected using Abbott's formula (Abbott 1925). All analyses were performed at 95% confidence interval using Minitab statistical software (Release 17, Minitab Inc., USA).

Results

Contact toxicity

Toxicity of Dichlorvos (positive control, DDVP 77%) insecticide was much greater compared to most potent essential oils (lowland zone, Kayonza) (Fig. 6). Among all tested essential oils, the essential oil of C. lusitanica from lowland zone showed the most potent contact toxicity than others with considerably low LD50 and LD90 values of 0.28 and 191.33 ppm, respectively in 6 h post treatment (Table 3). The least lethal effect was observed with the essential oil of C. lusitanica highland zone that resulted in lethal dose values (LD50 and LD90) of 0.64 and 706.21 ppm, respectively which were much higher than that produced by DDVP insecticide (0.008 and 1.03 ppm) within 6 h. Essential oil of C. lusitanica from midland zone exhibited moderate contact toxicity against houseflies with lethal dose values, LD50 of 0.41 ppm and LD90 of 453.24 ppm in 6 h of post treatment (Table 3).

Lethal dose (LD_{50}) values showed that the essential oil of *C. lusitanica* from lowland (Kayonza) exhibited higher toxicity to houseflies than other regions, but lower than toxicity produced by DDVP in 6 h, post treatment. However, the toxicity was not significant among all tested essential oils and also compared to Dichlorvos (DDVP 77%) insecticide. The LD₉₀ values showed the significant differences in toxicity against houseflies among all tested essential oils, and compared to DDVP insecticide, with exception to the toxicity of essential oil from lowland (Kayonza) that was not significant compared to the DDVP insecticide (P = 0.09).

In 12 h of post-treatment, the toxicity (LD₅₀) of essential oil from highland (Burera) against houseflies was significantly low compared to the oils from lowland (Kayonza) (P=0.00) and midland (Huye) (P=0.002) and Dichlorvos insecticide (P=0.00). The toxicity of essential oil from midland (Huye) was also lower compared to the toxicity of essential oil from lowland (Kayonza), though they showed no statistical difference (P=0.18). By taking into account

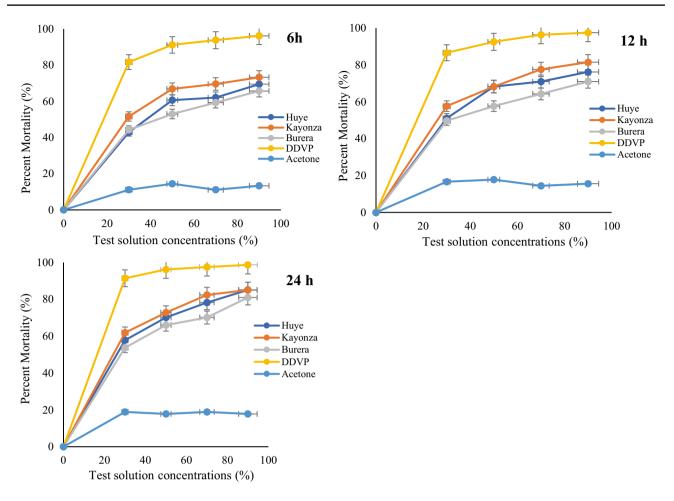


Fig. 6 Contact mortality percentages (mean ± SE) of adult houseflies exposed to Cupressus lusitanica essential oils and controls for 6 to 24 h

the LD_{90} values, the observed toxicities were not significantly different among all tested essential oils and DDVP insecticide, with the exception of oil from highland (Burera)

Table 3Contact toxicity ofCupressus lusitanicaessentialoils per studied ecologicalzones and DDVP against adulthouseflies in 6 to 24 h

that showed a significant lower toxic effect to adult houseflies compared to the DDVP insecticide (P = 0.00).

Treatment duration	EOs and DDVP treatments	LD ₅₀ (ppm)	LD ₉₀ (ppm)	Probit Model Equations
6 h	HLZ (Burera) MLZ (Huye) LLZ (Kayonza) DDVP	$\begin{array}{c} 0.64 \pm 0.20^{a} \\ 0.41 \pm 0.05^{a} \\ 0.28 \pm 0.06^{a} \\ 0.01 \pm 0.00^{a} \end{array}$	706.21 ± 33.98^{a} 453.24 ± 36.33^{b} 191.33 ± 5.71^{c} 1.03 ± 0.02^{c}	y = 0.39 x + 5.14 y = 0.44 x + 5.15 y = 0.44 x + 5.30 y = 0.61 x + 6.27
12 h	HLZ (Burera) MLZ (Huye) LLZ (Kayonza) DDVP	$\begin{array}{c} 0.34 \pm 0.03^{a} \\ 0.18 \pm 0.03^{b} \\ 0.12 \pm 0.02^{b} \\ 0.01 \pm 0.00^{c} \end{array}$	$\begin{array}{c} 176.37 \pm 26.95^{a} \\ 127.90 \pm 10.25^{ab} \\ 37.02 \pm 7.56^{ab} \\ 0.52 \pm 0.02^{b} \end{array}$	y = 0.46 x + 5.21 y = 0.45 x + 5.32 y = 0.60 x + 5.49 y = 0.65 x + 6.45
24 h	HLZ (Burera) MLZ (Huye) LLZ (Kayonza) DDVP	$\begin{array}{c} 0.24 \pm 0.07^{a} \\ 0.15 \pm 0.02^{ab} \\ 0.08 \pm 0.02^{bc} \\ 0.00 \pm 0.00^{c} \end{array}$	73.49 ± 11.14^{a} 22.01 ± 3.64 ^{ab} 16.26 ± 3.02 ^{ab} 0.19 ± 0.00 ^b	y = 0.51 x + 5.41 y = 0.68 x + 5.52 y = 0.51 x + 5.60 y = 0.60 x + 6.72

DDVP Dimethyl 2, 2-dichlorovinyl phosphate, LD50 and LD90=lethal concentrations that kill 50% and 90% of the exposed adult houseflies, respectively. Mean \pm SE of three replicates. Values followed by the same letter in column are not significantly different at P = 0.05 (Tukey's HSD test, one way-ANOVA). LLZ, MLZ and HLZ stand for lowland, midland and highland zones, respectively

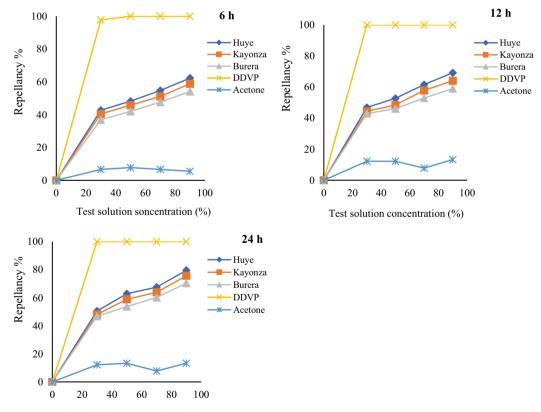
The comparison of LD₅₀ values obtained in 24 h showed that, the lethal effect of essential oil from lowland (Kayonza) was significantly higher compared to the oils from highland (Burera) (P = 0.03). Essential oil of C. lusitanica from midland (Huye) exhibited lower lethal effect than essential oil from lowland (Kayonza), but still was higher than the toxicity of essential oil from Burera against houseflies, though all showed no statistical difference (P > 0.05) in their toxicity against adult houseflies. DDVP insecticide showed a significant higher toxicity against houseflies compared to all tested essential oils, contrarily to the essential oil from Kayonza that showed no statistical difference in toxicity, but still low compared to the toxicity of Dichlorvos insecticide (P = 0.35). In consideration of lethal concentrations (LD₀₀), the difference in lethal effects was not significant among all tested essential oils and when compared to DDVP insecticide against houseflies, with exception to the essential oil of C. lusitanica from Burera that produced lower contact toxicity against houseflies compared to DDVP insecticide (P = 0.04).

Repellant activity

Current findings on repellency assay showed that adult houseflies were more tolerant at low concentrations of essential oils (30% v/v) and post-treatment time (6 h), but became more susceptible at higher concentration (90% v/v) and 24h post treatment time (Fig. 7).

The repellency rates of essential oils against adult houseflies were dose-exposure time-dependent with exception to Dichlorvos (DDVP 77%) insecticide, a positive control that showed 97.78% at test solution concentration of 30% v/v in 6 h of exposure time and 100% of repellencies at all tested concentrations within 24 h post treatment. High repellency potentials were observed for the essential oil of *C. lusitanica* from Huye followed by that from Kayonza and Burera at all tested concentrations of 30%, 50%, 70% and 90% v/v in 6 to 24 h of exposure (Fig. 7)

In 6 h of exposure time, all tested essential oils of *C. lusitanica* showed a significant higher Repellency against houseflies at concentration of 90% v/v, compared to the Repellency produced at 30% v/v (P = 0.008, 0.006, and 0.005 for Huye, Kayonza and Burera respectively) and 50% v/v (P = 0.045, 0.039 and 0.036 for Huye, Kayonza and Burera, respectively). However, there was no significant difference between repellencies produced at concentration of 90% and 70% v/v (P = 0.35, 0.27 and 0.31 for EOs from Huye, Kayonza and Burera, respectively). Repellencies of houseflies observed at essential oil concentration of 50% v/v were higher than that produced at 30% v/v for all tested essential oils, but still lower than repellency observed at 70%



Test solution concentration (%)

Fig. 7 Repellant activity of Cupressus lusitanica essential oils per studied ecological zones and controls against adult housefly in 6 to 24 h

v/v, however, all were not significantly different. Repellency percentages observed at 90% v/v was significantly higher than that produced at 30% v/v (P = 0.006, 0.05 and 0.02 for Huye, Kayonza and Burera, respectively) and at 50% v/v (P = 0.03 and 0.05 for Huye and Kayonza, respectively) after 12 h of post treatment with the exception of oils from Burera that showed no significant difference between their repellencies at concentrations of 90% and 50% v/v (P = 0.11). Although, it was higher than the repellency observed at 30% and 50% v/v, the repellency produced at 70% v/v showed no statistical difference for all tested essential oils.

Generally, after 24 h of exposure time, the oil's repellency percentage observed at concentration of 30% v/v were significantly lower than that observed at 90% v/v (P = 0.001, 0.00 and 0.002 for Huye, Kayonza and Burera, respectively) and at 70% (P = 0.03, 0.006 and 0.05 for Huye, Kayonza and Burera, respectively). The repellency observed at essential oil concentration of 50% v/v were also higher than the repellencies produced at 30% v/v with significance difference for essential oil from Kayonza (P = 0.047), but still lower than the repellency potentials observed at 70% v/v. Compare to the repellency produced at concentration of 90% v/v, the observed repellency at 50% v/v were significantly lower (P = 0.03, 0.004, and 0.017 for Huye, Kayonza and Burera, respectively).

Fumigant toxicity

Mortality of houseflies exposed to *C. lusitanica* essential oils in 6, 12 or 24 h are presented in Fig. 8, and corresponding lethal doses (LD_{50} and LD_{90}) are presented in Table 4.

Lethal concentrations (LD₅₀) showed that the toxic effects of essential oils against houseflies were significantly different among all tested essential oils and compared to the DDVP insecticide with the exception of oils from midland (Huye) that showed lower fumigant toxicity to houseflies compared to that produced by oil from lowland (Kayonza) (P = 0.63) in 6 h of exposure time (Table 2). Lethal dose (LD₉₀) values obtained in 6 h of exposure showed a significant difference in

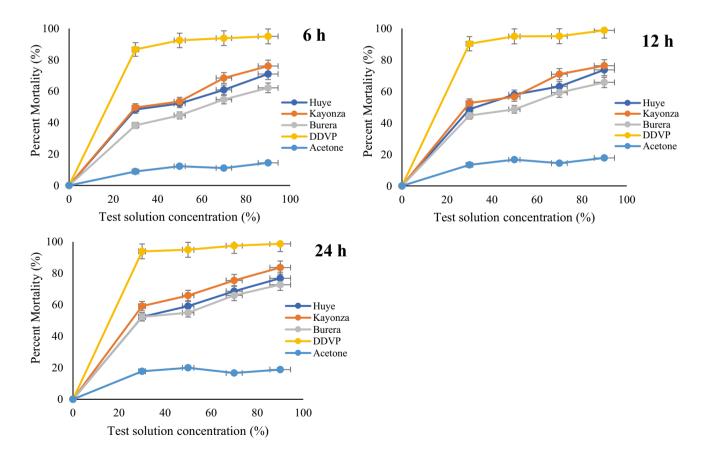


Fig.8 Fumigant mortality (mean \pm SE) of adult houseflies exposed to essential oils of *Cupressus lusitanica* from studied ecological zones and controls for 6 to 24 h

Table 4Fumigant toxicity ofessential oils of Cupressuslusitanica per studied ecologicalzones and DDVP against adulthouseflies in 6 to 24 h

Treatment duration	EOs and DDVP treatments	LD ₅₀ (ppm)	LD ₉₀ (ppm)	Probit Model equations
6 h	HLZ (Burera) MLZ (Huye) LLZ (Kayonza) DDVP	$\begin{array}{c} 1.90 \pm 0.18^{a} \\ 0.66 \pm 0.09^{b} \\ 0.51 \pm 0.05^{b} \\ 0.01 \pm 0.00^{c} \end{array}$	$\begin{array}{c} 1250.21 \pm 77.18^{a} \\ 521.36 \pm 70.00^{b} \\ 111.43 \pm 5.13^{c} \\ 0.52 \pm 0.03^{c} \end{array}$	y = 0.46 x + 4.85 y = 0.44 x + 5.10 y = 0.55 x + 5.16 y = 0.83 x + 6.51
12 h	HLZ (Burera) MLZ (Huye) LLZ (Kayonza) DDVP	$\begin{array}{c} 0.81 \pm 0.18^{a} \\ 0.39 \pm 0.04^{b} \\ 0.29 \pm 0.03^{bc} \\ 0.00 \pm 0.00^{c} \end{array}$	$\begin{array}{c} 766.80 \pm 45.18^{a} \\ 159.35 \pm 14.14^{b} \\ 81.47 \pm 8.03^{b} \\ 0.36 \pm 0.01^{b} \end{array}$	y = 0.45 x + 5.04 y = 0.49 x + 5.19 y = 0.49 x + 5.29 y = 0.66 x + 6.58
24 h	HLZ (Burera) MLZ (Huye) LLZ (Kayonza) DDVP	$\begin{array}{c} 0.38 \pm 0.05^{a} \\ 0.28 \pm 0.03^{a} \\ 0.15 \pm 0.01^{b} \\ 0.00 \pm 0.00^{c} \end{array}$	$220.32 \pm 31.51^{a} \\ 80.65 \pm 4.20^{b} \\ 24.79 \pm 3.48^{bc} \\ 0.20 \pm 0.17^{c}$	y = 0.46 x + 5.20 y = 0.50 x + 5.31 y = 0.57 x + 5.49 y = 0.60 x + 6.70

DDVP Dimethyl 2, 2-dichlorovinyl phosphate, LD_{50} and LD_{90} =lethal concentrations that kill 50% and 90% of the exposed adult houseflies, respectively. Mean ± SE of three replicates. Values followed by the same letter in *column* are not significantly different at *P*=0.05 (Tukey's HSD test, one way-ANOVA). LLZ, MLZ and HLZ stand for lowland, midland and highland zones, respectively

toxicity among all tested essential oils and compared to DDVP insecticide, except for the oils from lowland (Kayonza) compared to DDVP insecticide (P = 0.61). LD₅₀ values revealed higher fumigant toxicity of essential oil from lowland (Kayonza) to houseflies compared to the oil from highland (Burera) (P = 0.006) in 12 h. Although the fumigant effect was lower and not significant compared to the oil from lowland (Kayonza) (P = 0.79), the essential oil from midland (Huye) exhibited higher fumigant toxicity than that from highland (Burera) (P=0.02). By comparing lethal concentrations (LD₉₀), the essential oil of C. lusitanica from highland (Burera) produced a significantly lower fumigant toxicity to houseflies than the oil from other tested essential oils and DDVP insecticide (P < 0.05). Moreover, the fumigant toxicity produced by essential oil from lowland (Kayonza) to houseflies was not significantly different from the oil from midland (Huye) (P=0.55) and DDVP insecticide (P = 0.51).

Significant difference in fumigant toxicity were observed among all tested essential oils of C. lusitanica against adult houseflies in 24 h of exposure time (P < 0.05), with the highest toxic effect being observed for the essential oil from lowland (Kayonza), followed by the essential oil from midland (Huye) that exhibited insignificantly higher toxic effect compared to the oil from highland (Burera) (P = 0.06). Fumigant toxicity produced by all tested essential oils were significantly lower compared DDVP insecticide (P < 0.05). LD₉₀ values proved a statistical difference in fumigant toxicity to adult houseflies among all tested essential oil from C. lusi*tanica* and DDVP insecticide (P < 0.05) with the exception of the essential oil from lowland that showed no statistical difference in toxic effect against houseflies compared to the essential oil of C. lusitanica from midland (P = 0.18) and DDVP insecticide (P = 0.75).

Discussion

Present study clearly indicated that the C. lusitanica essential oils were highly toxic to adult houseflies and the results are supported by different reports on insecticidal activities owned by different species of Cupressaceae family against houseflies and other household pests. A study conducted by Elbermawy et al. (2011) showed the repellent potential and larvicidal activity of the essential oil of Cupressus sempervirens towards larvae of houseflies, while Yang et al. (2020) observed higher contact toxicity ($LD_{50} = 1.23 \text{ g/cm}^2$) and lower fumigant toxicity (LD₅₀=556.80 g/cm²) of essential oil from C. sempervirens against stored product pest (Sitophilus zeamais Motschulsky). The findings of Lee et al. (2015) confirmed the strong repellent behavioral responses of fruit flies and houseflies to the essential oil from Hinoki cypress (Chamaecyparis obtusa) after 5 h of exposure, while the oil of Juniperus communis, Juniperus virginiana and Thuja occidentalis (Cupressaceae family) were also reported to exhibit lethal effects on houseflies in 24 h with lethal doses (LD₅₀) of 86.0, 24.0 and 42.0 μ g/ fly after topical application and 10.80, 80.0 and 6.30 µg/cm³ after fumigant test, respectively (Pavela 2008). In addition, among eight Cupressus species investigated by Giatropoulos et al. (2013), the essential oil of Cupressus benthamii showed potent toxicity (LC₅₀=37.5 mg/L) against houseflies with the major plant constituents being umbellulone, limonene, δ -3carene and α -pinene.

Observed toxicity and repellency potentials of *C. lusitanica* essential oils varied as per ecological zone, and increased with the concentrations of oils. Despite not being tested directly, the insecticidal potentials of *C. lusitanica* essential oil in current study could be linked to the presence of previously reported individual major chemical components such as

y-terpinene, umbellulone, Bornyl acetate, linalool, myrcene, limonene. α -pinene and sabinene that were found in essential oil of C. lusitanica from lowland (Kayonza) (Table 4), which exhibited high contact (Fig. 5) and fumigant toxicity (Fig. 7) against adult houseflies. The higher repellency potential to adult houseflies was observed for the essential oil of C. lusitanica from midland zone (Huye) that was dominated by δ -3-Carene, Sabinene, α -Pinene, α - Terpinene, Umbellulone, Camphene hydrate, α-Terpineol, 1,8-Cineole and Linalool, whereas the essential oils from highland zone (Burera) mainly contained Sabinene, Myrcene, α -Pinene, δ -3-Carene, Linalool and Umbellulone (Fig. 6). Current results on insecticidal activity of C. lusitanica oils are in agreement with a number of studies that have investigated the insecticidal activity of essential oils' components against houseflies (Bett 2015; Giatropoulos et al. 2013; Langsi et al. 2018; Teke et al. 2013; Palacios et al. 2009) reported low and moderate fumigant toxicity of α -pinene and β -pinene against adult *M*. domestica. Toxic and repellant effects of the terpenes such as α - pinene, β -pinene, limonene, linalool, myrcene, α -terpineol, terpinen-4-ol, α -, γ -terpinene, 1,8-cineole, bornyl acetate and terpinolene against houseflies have been demonstrated (Zhang et al. 2017; Haselton et al. 2015). The study conducted by Urzúa et al. (2010) demonstrated the moderate toxicity of α -pinene and limonene against houseflies (*M. domestica*) with lethal concentrations (LC50) of 12.10 and 5.0 mg/dm³ in 30 min, respectively. Terpinolene, p-cymene and other 11 monoterpenes exhibited strong fumigant activity against *M. domestica* in the study conducted by Zhang et al. (2017), while El-Sherbini and Osman (2014) reported the variable mortality of houseflies from 65 to 100%, 55 to 100% and 75 to 100% in 6 to 24 h of exposure to monoterpenes; α -pinene, myrcene and limonene, respectively. Haselton et al. (2015) showed that the α -pinene was the antenna-stimulatory compound and it exhibited the baseline repellent properties against houseflies under laboratory conditions. Moreover, the study conducted on the insecticidal activity of essential oils' components including limonene, y-terpinene, linalool, verbenone and camphor demonstrated the moderate toxic effect against adult female houseflies via topical application with lethal doses of 226.63, 236.47, 238.05, 426.67 and 512.12 µg/fly in 24 h, respectively, and 213.36, 221.55, 209.73, 409.93 and 477.91 µg/fly in 48 h, respectively (Tian 2017). In the same study, p-cymene showed a significant repellency at low concentration (0.1 μ g/ μ L), while limonene, γ -terpinene and linalool exhibited significant repellency against houseflies (M. domestica) only at higher concentrations (10 and 100 μ g/ μ L).

In most cases, the insecticidal constituents of many plant extracts and essential oils are monoterpenoids due to their anti-cholinesterasic properties which cause high level of mortality of insects at higher concentrations (Chen et al. 2018). Therefore, the chemical composition of essential oil in current study exhibited toxic and repellent effects on adult houseflies in one way or another; either the active compounds act individually or in synergy. However, this assumption was not verified during this study.

Conclusion

Bioassay results showed a promising insecticidal potential of *C. lusitanica* essential oils against adult houseflies that varied from one region to another with higher fumigant and contact toxicity being exhibited by the essential oil from the leaves collected in lowland (Kayonza) followed by that from midland (Huye), while least toxicity (fumigant and contact) was produced by oils from highland (Burera). On the other hand, higher repellency capacity was exhibited by essential oils of *C. lusitanica* collected from midland (Huye), while essential oil of plant from highland (Burera) showed the least repellency capacity against adult houseflies. The observed variation in insecticidal activities of essential oils against adult housefly is obviously due to unequal distribution of chemical composition of *C. lusitanica* essential oils within studied agro-ecological zones.

Essential oils of *C. lusitanica* provide a safer prophylactic measure for the control of adult housefly population. However, the future study could look into its toxicity and efficacy at each stage of housefly development. Moreover, further investigation is needed through a fractionation of all essential oil components and conducting individual biological tests prior to identification of the real active ingredients.

List of abbreviations AEZs: Agro-ecological zones; *C. lusitanica: Cupressus lusitanica* Mill.; DDVP: Dimethyl 2, 2-dichlorovinyl phosphate; EOs: Essential oils; FTIR: Fourier transform infrared spectroscopy; GC-MS: Gas chromatography-mass spectrometry; HLZ: highlands zone; LD_{50} : lethal concentration that kills 50% of housefly population in assay; LD_{90} : lethal concentration that kills 90% of housefly population in assay; LLZ: lowlands zone; MLZ: midlands zone; PR: Repellency percentage

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Authors' contributions PN, JC, JM and TM designed the research study. PN collected samples, performed the analytical works and analyzed the data as well as writing of first draft of the Manuscript. JC, JM and TM supervised the research study. All authors revised and approved the final manuscript.

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Declarations

Ethics approval and consent to participate This study was approved by the Department of Chemistry and Biochemistry, Moi University, Kenya (Approval No. MS/ACH/4317/20).

Consent for publication Not applicable.

Competing interests The authors declare no conflict of interest exist.

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