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Adulticidal and fecundity effects of essential oils of *Cymbopogon citratus* and *Tagetes minuta* against *Phlebotomus duboscqi*

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Abstract
Background: The efficacy of *Cymbopogon citratus* and *Tagetes minuta* essential oils in killing and fecundity of the adult females of the sand fly *Phlebotomus duboscqi* was tested in the laboratory.
Materials and Methods: 1.0ml of each essential oil extract at concentrations of 0.125; 0.250; 0.500; 0.750 and 1mg/ml and the controls, DEET and Tween 80 were applied to the inner surface and bottom of a sterile pot and thirty adult *P. duboscqi* specimens aspirated into the pots. The parameters observed were insect mortalities after 24, 48 and 72 h as well as the number of eggs obtained from females subjected to the different treatments.
Results: The results showed that both *T. minuta* and *C. citratus* oils were highly potent against *P. duboscqi* sandflies with mortality rates of 100.00 and 82.22 % on female sandflies and 100.00 and 88.89 % on male sandflies respectively after 72h. Between the two oils, *C. citratus* was significantly more potent ($P < 0.05$) and caused higher mortality than *T. minuta* against both male and female sandflies. However, there was no statistical difference in mortality rates between males and females subjected to each of the two oils at 24 h, 48 h and 72 h ($P > 0.05$). Female sandflies treated with the oil of *C. citratus* oil had significantly lower mortality rates than those laid by sand flies treated with *T. minuta* oil ($P < 0.05$; $P = 0.00084$).
Conclusion: I conclude that the two essential oils are promising natural insecticides due to their safety advantage over chemical insecticides. I do recommend further studies in the field using human subjects before their adoption for use against Phlebotomine sandflies.

Keywords: *Phlebotomus duboscqi*, sandfly, *Tagetes minuta*, *Cymbopogon citratus,* Leishmaniasis.

1. Introduction
The blood-feeding females of phlebotomine sand flies (Diptera: Psychodidae: Phlebotominae) are usually considered to be the only natural vectors of protozoan *Leishmania* species (Euglenozoa: Trypanosomatidae), the causative agents of the neglected tropical disease leishmaniasis [1, 2]. Of approximately 900 sand fly species, no more than 70 have been implicated in leishmaniasis transmission [3, 4, 5]. Even fewer have been associated with *Phlebovirus* and other arboviruses of biomedical importance [3, 4, 6] and only one (*Lutzomyia verrucarum* sensulato) is the vector of the alphaproteobacterium *Bartonella bacilliformis*, which causes Carrion's disease in a limited Andean region [7, 8].
The World Health Organization (WHO) estimates that over 2.3 million new cases of leishmaniasis occur each year and that, at least 12 million people are presently infected worldwide [9] (World Health Organization Leishmaniasis Homepage). In Kenya, phlebotomine sandflies transmit both visceral and cutaneous leishmaniases. Visceral leishmaniasis (VL), caused by *Leishmania donovani* is transmitted by *Phlebotomus martini* (Diptera: Psychodidae) [10, 11]. On the other hand, *Phlebotomus duboscqi* sandflies transmit *L. major,* one of the causative agents of cutaneous leishmaniasis (CL) [12]. The current management strategy for leishmaniasis in Kenya is mainly based on chemotherapy for treatment of infected cases and use of insecticides in vector control to reduce transmission [13, 14].
Vector control using insecticides has been recommended by the World Health Organization [15]. However, acquired resistance and environmental pollution due to the repeated application of persistent synthetic insecticides have led to increased interest in new natural chemicals [16]. In addition, usage of highly persistent and toxic synthetic insecticides has led to development of resistance in vector populations. Further, environmental pollution due to the repeated applications is a challenge. Thus, the harmful side effects of these chemicals on both animals

and humans have progressively limited their usage and have led to increased interest in alternative new natural chemicals that are environmentally safe, affordable and effective in management of leishmaniases. In this context, screening of natural products has received the attention of researchers around the world. Since many diseases that are transmitted by insects such as malaria, dengue fever, yellow fever, leishmaniasis and Chaga's disease are endemic in developing countries; the search for insecticides and repellents of botanical origin has been driven by the need to find new products that are effective, but also safer and more affordable than currently available products [17].

In recent years, the use of essential oils (EOs) derived from aromatic plants as low-risk insecticides has increased considerably owing to their popularity with organic growers and environmentally conscious consumers [18]. EOs are easily produced by steam distillation of plant material and contain many volatile, low-molecular-weight terpenes and phenolics. EOs have repellent, insecticidal, and growth-reducing effects on a variety of insects [19]. They have been used effectively to control preharvest and postharvest phytophagous insects and as insect repellents for biting flies and for home and garden insects. The compounds exert their activities on insects through neurotoxic effects involving several mechanisms, notably through GABA, octopamine synapses, and the inhibition of acetylcholinesterase. With a few exceptions, their mammalian toxicity is low and environmental persistence is short.

Essential oils of an appreciable number of plants have been shown to be repellent against various haematophagous arthropods [20, 21]. Lemongrass, *Cymbopogon* spp. produce the most used natural repellents in the world [22]. For example, essential oils from *Cymbopogon martinii martinii* elicited 100% repellency against *Anopheles* sp. mosquitoes in field tests for 12 hours [23]. Essential oil of *Cymbopogon winterianus*, mixed with 5% vanillin, gave 100% repulsion against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles dirus* for 6 hours [24]. Lemongrass, *Cymbopogon citratus* essential oil is obtained from the aerial parts of the plant. The plant has been widely recognized for its enthnobotanical and medicinal usefulness [25]. Other documented effects of essential oils of plants include insecticidal [26-31] antifungal [32], antimicrobial [33, 34], and the therapeutic properties [25]. However, there are relatively few studies that have been carried out to determine the efficacy of essential oils from citronella as arthropod repellents [35] and specifically against sandflies.

On the other hand, the essential oil of the Mexican marigold, *Tagetes minuta* L., has beenshown to have both larvicidal and adulticidal effects on mosquitoes [36-38]. The active components were isolated from different parts of the plant. Green *et al*. [36] reported mosquito larvicidal activity in the extract of *Tagetes minuta* flowers. Perich *et al*. [37] compared biocidal effects of the whole-plant extracts of three *Tagetes* spp. and showed that, *T. minuta* had the greatest biocidal effect on the larvae and adults of *Ae. aegypti* (L.) and *Anopheles stephensi* (L). Bioassays carried out with simultaneous steam distillates of *T. minuta* flowers showed 90% larval mortality at lethal concentrations (LC_{90}) of 4 and 8 ppm and against the adult at 0.4 and 0.45% against *Aedes aegypti* and *Anopheles stephensi*, respectively [37]. Recently, Ireri *et al*. [39] demonstrated that, methanol and ethyl acetate crude extracts of *T. minuta* derived from the aerial parts had significant mortality against both male and female *P. duboscqi*, Neveu Lemaire (Diptera:Psychodidae). Further, Mong'are *et al*. [40] found that, similar crude extracts reduced the fecundity of *P. duboscqi* by 53%. No similar work has been reported for *C. citratus* and the responsible compounds have not been identified for both *T. minuta* and *C. citratus*.

With regard to the foregoing, the present study sought to identify the constituent compounds and evaluate the insecticidal effects of the essential oils of the lemon grass, *C. citratus* and *T. minuta* against adult sandflies, *P. duboscqi*.

2. Materials and methods

2.1 Collection of plant materials

Fresh leaves of the lemon grass, *Cymbopogon citratus* were collected from the equatorial rainforest in Kakamega, Kenya. The plant identity was confirmed by a taxonomist and a voucher specimen was deposited at KEMRI's Center for Biotechnology Research and Development (CBRD) for future reference. The leaves were screened and dry and/or damaged ones were discarded. The remaining good leaves were used for extraction while still fresh. On the other hand, floral and foliar parts of *T. minuta* plants were collected from Marigat District of Baringo County, Rift Valley region, Kenya. The plant parts were packed in a cold box and transported to the International Centre for Insect Physiology and Ecology (*icipe*), Kasarani, Nairobi, Kenya where extraction of the essential oils was done. The plant identity was also confirmed by a taxonomist and a voucher specimen was deposited at KEMRI's Center for Biotechnology Research and Development (CBRD) for future reference.

2.2 Extraction of essential oils of *Tagetes minuta* and *Cymbopogon citratus*

Extraction of the essential oil of the lemon grass *c. citratus* was done as described by Adeniran and Fabiyi [41]. The fresh leaves were immersed in distilled water after which they were subjected to steam distillation. The mixture of steam and the volatile oil generated was passed through a condenser and collected in a flask. Then, a separating funnel was used to separate the oil from water. The recovered oil was dried *using* anhydrous sodium sulphate and kept in a refrigerator at 4 ° C for subsequent use [41].

For the extraction of the essential oil from *T. minuta*, fresh plant material was sliced and hydro-distilled by using a Clevenger-type apparatus [42], with slight modifications [43]. Heat was provided by a heating-mantle equipped with a thermostat and the temperature maintained at 90 °C. The plant material was immersed in distilled water then placed into a 2 litreround-bottomed flask and hydro-distilled for 2 hours. The distillate was collected as the essential oil band above the water [44].

2.3 Gas chromatography and mass spectrometry analysis of essential oil of *Tagetes minuta* and *Cymbopogon citratus*

The analysis of the essential oils was carried out in the Behavioural and Chemical Ecology Dept. laboratory at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi. Samples essential oil of each of the two plants were diluted in high purity dichlomethane (99.9%, Sigma, Aldrich) analyzed on a coupled GC-MS using a Hewlett Packard (HP) 7890 Series A gas chromatograph (Agilent technologies, Wilmington, DE, USA) coupled to a 5975 C Series mass spectrometer fitted with an 7683 B Series autosampler (Agilent technologies, Wilmington, DE, USA) and a triple axis detector [45]. The GC is equipped with a non-polar capillary column (HP5 MS 5% with phenylmethyl silicone) that was 30 m (length × 0.25 µm (i.d.) and 0.25 µm (film

thickness) for the separation of the chromatographic peaks. The GC is also coupled to a HP monitor (L1710) for displaying of the chromatographic data which will be acquired and studied using the 3365 MSD ChemStation software (G1701Ea E.20.00.493).

Samples were injected in the split mode at a ratio of 1:10 – 1:100. The injector was kept at 250°C and the transfer line at 280°C. The column was maintained at 50°C for 2 min and then programmed to 260°C at 5°C/min and held for 10 min at 260°C. The MS was operated in the EI mode at 70 eV, in m/z range 42-350. Identification of the compounds was performed by comparing their retention indices and mass spectra with those found in literature [46] and supplemented by Wiley and QuadLib 1607 GC-MS libraries. The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalization, all relative response factors being taken as one [32].

2.4 Sand fly colony maintenance

Sandflies were obtained from a colony of *P. duboscqi* Neveu Lemaire that originated from Marigat Division, Baringo district, Rift Valley, and were maintained at the Centre for Biotechnology Research and Development (CBRD) insectaries in Kenya Medical Research Institute, Nairobi. The colony of *P. duboscqi* was established using field-captured females that were held in cages and maintained according to the methods of Beach *et al.* [47] with some modifications. Briefly, female sandflies were fed on blood using Syrian golden hamsters that were anaesthetized with sodium pentobarbitone (Sagatal®). The hamsters' under bellies were usually shaved using an electric shaver for easy access for feeding by sandfly. The sandflies were reared at 28 ± 1°C, and an average RH of 85-95% and 12:12 h (light: dark) photoperiod in Perspex® insect rearing cages. Sandflies were fed *ad libitum* on slices of apple that were supplied daily as a source of carbohydrates.

2.5 Experimental set up

Each oil concentration (1.0 ml), and the controls, DEET and Tween 80 were applied to the inner surface and bottom of each pot using a pipette. Thirty adult *P. duboscqi* flies (15 males and 15 females) were placed inside of the pots after the application of the oils, and the concentrations that were used were from 0.125; 0.250; 0.500; 0.750 and 1mg/ml of *C. citratus* and *T. minuta* essential oils. In this experiments, the parameters observed were insect mortality after 24, 48 and 72 h, mortality rate differences between female and male insects and the number of eggs obtained from females subjected to the oils;

The percentage mortality was calculated by using the formula below;

$$\text{Percent mortality} = \frac{\text{Number of dead adults}}{\text{Number of adults introduced}} \times 100$$

The corrections for mortality when necessary were done using Abbot's (1925) formula

$$\text{Corrected percentage mortality} = \frac{\text{\% Kill in treated - \% kill in control}}{\text{100 - \% Kill in control}} \times 100$$

2.6 Data analysis

All experiments were done in replicates. Data on adult mortality was recorded using the Microsoft Excel programme. Control groups in the experimental bioassays with >20% mortality were repeated. Where mortality in the control groups fell between 5 and 20%, the observed mortality was corrected using Abbott's formula [48]. The dose mortality data was analysed by log-probit method of Finney [49] and lethal concentrations for 50% (LD_{50}) and 90% (LD_{90}) determined. Statistical significance of the recorded mortality of the various test concentrations and the controls were analyzed using one-way analysis of variance (ANOVA) at $P \leq 0.05$.

3. Results

3.1 Chemical composition of *Cymbopogon citratus*

The volatile Lemon grass essential oil obtained from hydro distillation had the usual light yellow colour, a lemony scent, and an extraction yield of 0.6% (v/w) when distilled from the fresh aerial parts of the plant, as was done in the present study. Thirty compounds which constituted 98.28% of the total oil were identified. The constituents identified by GC-MS analysis, their retention times and area percentages are summarized in Table 1. The oil was dominated by monoterpene hydrocarbons. This monoterpene fraction was characterized by a high percentage of Geranial (20.45%), Myrcene (14.24%), Neral (11.57%), and Verbenene (9.26%) among others.

Table 1: Chemical composition of *C. citratus* essential oil identified by GC-MS

S. No.	Compound	RT	Area Pct
1	1-methyl-1,3-Cyclohexadiene	5.57	3.76
2	3-methylene-Cyclohexene	5.95	0.14
3	Myrcene	11.15	14.24
4	1,3,8-Menthatriene	11.39	7.20
5	alpha-Terpinene	11.55	0.19
6	Verbenene	11.75	9.26
7	(Z)-beta-ocimene	11.93	1.28
8	(E)-beta-ocimene	12.11	1.26
9	gamma-Terpinene	12.31	0.11
10	para-Cymenene	12.89	6.42
11	Terpinolene	13.30	3.66
12	allo-Ocimene	13.47	0.61
13	2,6-dimethyl-1,3,5,7-octatetraene	13.63	1.54
14	2,3,5-Trimethyl-2,3,5-hexanetricarbonitrile	13.77	1.55
15	trans-Chrysanthemal	13.88	0.60
16	(Z)-Isocitral	14.08	1.84
17	Trans-p-Mentha-2 8-dienol	14.37	6.65
18	5-isopropyl-2-methyl-cyclopent-1-enecarbaldehyde	14.95	1.61
19	Citronellylformate	15.13	0.42
20	Neral	15.33	11.57
21	delta-3-Carene	15.54	0.69
22	Geranial	15.85	20.45
23	2-Undecanone	16.01	1.04
24	thuj-3-en-10-al	16.36	0.14
25	Piperitenone	16.74	0.20
26	2-methyl-3-phenyl-propanal	17.53	0.16
27	3,5-Heptadienal, 2-ethylidene-6-methyl-	17.71	0.39
28	Z-Caryophyllene	17.80	0.17
29	(Z)-alpha-Bergamotene	17.93	0.11
30	2-Tridecanone	18.60	1.02
Total			98.28

3.2 Chemical composition of *Tagetes minuta*

The GC-MS analysis of the distillate of the aerial parts of *T. minuta* revealed that the oil is rich in terpenes. A total of 29 compounds were identified representing 98.95 % of the total oil composition, as presented in Table 2. The major components of the essential oil were Dihydro-Tagetone

(21.15%), (E)-Tagetone (16.21%), (Z)-Tagetone (14.99%), (Z)-beta-Ocimene (9.84%), Limonene (7.40%), allo-Ocimene (6.69%) and (Z)-Ocimenone (4.12%). Oxygenated monoterpenes were the most abundant chemical class of compounds in the essential oil.

Table 2: Chemical composition of *T. minuta* essential oil identified by GC-MS

S. No.	Compound	RT	Area Pct
1	Ethyl 2-methylbutanoate	7.94	0.31
2	Pentanoic acid, ethyl ester	8.03	0.16
3	1-butanol 2-methyl- acetate	8.66	0.42
4	alpha-Thujene	9.69	0.65
5	alpha-Pinene	9.82	0.43
6	Camphene	10.14	0.51
7	Sabinene	10.67	1.17
8	Myrcene	11.03	0.62
9	alpha-Phellandrene	11.28	1.06
10	alpha-Terpinene	11.53	0.58
11	Limonene	11.79	7.40
12	(Z)-beta- Ocimene	11.97	9.84
13	Dihydro-Tagetone	12.33	21.15
14	2-Cyclohexen-1-one, 5-methyl-2-(1-methylethyl)-	12.89	2.33
15	allo-Ocimene	13.52	6.69
16	(E)-Tagetone	13.88	16.21
17	(Z)-Tagetone	14.01	14.99
18	Borneol	14.21	0.53
19	2-propenal,2-methyl-3-phenyl-	14.91	1.30
20	(Z)-Ocimenone	15.13	4.12
21	Car-3-en-2-one	15.24	2.81
22	Thymol	15.96	0.53
23	Piperitenone	16.72	1.79
24	(E)-Caryophyllene	17.80	0.62
25	Aromadendrene	18.04	0.55
26	alpha-Humulene	18.24	0.72
27	Germacrene D	18.58	0.38
28	Bicyclogermacrene	18.76	0.73
29	delta-Cadinene	19.05	0.35
Total			98.95

Insecticidal effects of the essential oils of *C. citratus* and *T. minuta* on adults of the sandfly, *P. duboscqi* 24, 48 and 72 h after treatment are shown in Tables 3–5. Also, the number of eggs laid by female flies during the same period is included. Among the two oils, that of *C. citratus* was significantly ($P < 0.05$) more potent and caused higher mortality than that of *T. minuta* on against both male and female sand flies. The results show that, after 24 h, treatment with the oil of *C. citratus* at a concentration of 1 mg/ml caused a mortality of 91.11 and 88.89 % against female and male sandflies, respectively. However, the essential oil of *T. minuta* at the same concentration, recorded a relatively lower mortality of 71.11% 66.67 % in female and male sand flies, respectively. The results of this study demonstrate that, the effects of the oils were dose-dependent and increased with the concentration of the oil. The low concentrations tested inflicted low levels of mortality. This is clearly evident for all the concentrations tested with the lowest one (0.125 mg/ml) of *C. citratus* and *T. minuta* oils causing 51.11 and 28.89% mortality, respectively. Further, the mortality levels recorded also increased with time. Thus, the highest mortality levels were observed at 72 h after treatment for all the concentrations tested. In fact, after 72 h after treatment, the essential oils of *C. citratus* and *T. minuta* at a concentration of 1 mg/ml recorded a mortality of 100.00 and 82.22 % respectively, on female sandflies. At the same concentration, *C. citratus* and *T. minuta* oils caused mortalities of 100.00 and 88.89% respectively, in male sandflies. There was no statistical difference in mortality rates between males and females subjected each of the two oils *C. citratus* and *T. minuta* at 24 h, 48 h and 72 h ($P > 0.05$). However, there was a significant difference between the mortality rates of *C. citratus* and *T. minuta* ($P < 0.05$) observed for both male and females after 24 h ($P=0.00014$), 48 h ($P=0.0000238$) and 72 h (0.00084). The LD_{50} values for *C. citratus* and *T. minuta* oils were 0.07mg/ml and 0.2 mg/ml respectively.

Table 3: Cumulative mortality (mean percentage ± S.D.) of essential oils of *C. citratus* and *T. minuta* in the first 24 h on adults of *Phlebotomus duboscqi*

	Concentration (mg/ml)	Mortality (%) *C. citratus*			Mortality (%) *T. minuta*		
		% males	% females	No. of eggs laid	% males	% females	No. of eggs laid
	0.125	44.44±0.58	51.11±0.58	11.33±5.86	26.67±1.00	28.89±0.58	22.67±7.51
	0.25	62.22±1.53	64.44±1.15	7.67±4.51	35.56±0.58	37.77±0.58	17.33±3.51
	0.50	68.89±2.08	75.56±1.15	6.33±3.79	42.22±0.58	42.22±1.53	12.67±5.13
	0.75	86.67±1.00	82.22±0.58	4.67±2.52	51.11±1.53	53.33±1.73	9.33±3.51
	1.00	88.89±1.15	91.11±0.58	2.33±1.53	71.11±0.58	66.67±1.00	6.67±1.52
DEET	0.196	100±0.00					
Tween 80	53.4	2.22±0.58					

Table 4: Cumulative mortality (mean percentage ± S.D.) of essential oils of *C. citratus* and *T. minuta* in the first 48 h on adults of *Phlebotomus duboscqi*

	Concentration (mg/ml)	Mortality (%) *C. citratus*			Mortality (%) *T. minuta*		
		% males	% females	No. of eggs laid	% males	% females	No. of eggs laid
	0.125	75.56±0.58	66.67±1.00	19.67±9.07	46.67±1.00	40.00±0.00	39.67±3.51
	0.25	80.00±0.58	75.56±0.58	12.67±4.51	44.44±1.53	42.22±1.15	33.67±13.43
	0.50	84.44±0.58	84.44±0.58	9.33±1.53	51.11±0.58	53.33±1.00	25.67±1.79
	0.75	95.56±0.58	88.89±0.58	6.67±1.155	55.56±1.52	57.78±1.15	18.33±2.89
	1.00	97.78±0.58	100.00±0.00	3.33±0.58	75.56±0.58	73.33±1.00	12.33±1.53
DEET	0.196	100.00±0.00					
Tween 80	53.4	4.44±0.58					

Table 5: Cumulative mortality (mean percentage ± S.D.) of essential oils of *C. citratus* and *T. minuta* in the first 72 h on adults of *Phlebotomus duboscqi*

	Concentration (mg/ml)	Mortality (%) *C. citratus*			Mortality (%) *T. minuta*		
		% males	% females	No. of eggs laid	% males	% females	No. of eggs laid
	0.125	84.44±0.58	68.89±0.58	29.00±7.94	57.78±0.58	44.44±0.58	51.00±5.57
	0.25	88.89±0.58	77.78±0.58	21.33±4.93	64.44±1.52	46.67±1.00	36.67±19.60
	0.50	93.33±1.00	95.56±0.58	11.00±2.66	73.33±1.00	62.22±0.58	22.00±10.58
	0.75	97.78±0.58	97.78±0.58	7.67±1.53	77.78±0.58	75.56±0.58	23.67±4.51
	1.00	100.00±0.00	100.00±0.00	3.33±0.58	88.89±0.57	82.22±0.58	18.67±8.62
DEET	0.196	100±0.00					
Tween 80	53.4	6.66±0.00					

With regard to the number of eggs that were laid by female sandflies that were treated with the essential oils, those treated with the oil of *C. citratus* oil were significantly lower than those laid by sand flies that were treated with that of *T. minuta* oil ($P< 0.05$; $P= 0.00084$). In comparison with the controls, flies subjected to Tween 80 which was a negative control laid significantly higher ($P> 0.05$) number of eggs than those treated with the essential oils of *C. citratus* and *T. minuta*.

4. Discussion

There are many reports on the chemical composition of the oils from the plants belonging to the species *C. citratus* [22, 50, 51, 52, 53, 54, 55, 56]. Most of these reports indicate that neral and geranial are the main characteristic constituents of *C. citratus* [57]. The results of the GC-MS analysis of *C. citratus* obtained for this study concur with previous studies by Matasyoh *et al.* [32] which demonstrated that the oil was dominated by monoterpene hydrocarbons which accounted for 94.25% of the oil. In the study, the monoterpene fraction was characterized by a high percentage of geranial (39.53%), neral (33.31%), myrecene (11.41%) and geraniol (3.05%). Only 0.78% of the components identified were sesquiterpenes [32]. In other studies, Farhang *et al*. [58] identified α- citral (39.16 %), Z- citral (30.95 %), limonene (5.83 %), caryophyllene (3.44 %) and ceranyl acetate (3.1 %) as the main components in *C. citratus* essential oil. Gupta *et al.* [59] found that the main components of *C. citratus* essential oil were dominated by citral (77.8%), limonene + traces of eucalyptol (4.0%), geraniol (2.7%), 6 methyl-5-hepten-2-one (2.4%) and geranyl acetate (1.1%). The differences in the composition of the essential oil might have been derived both from harvest time and local, climatic and seasonal factors, or it may be hypothesized that these samples belong to a different chemotype [60].

The qualitative and quantitative analyses of the essential oil extract obtained from *T. minuta* in this study showed that there are six major components in the extract. The major components of the essential oil were Dihydro-Tagetone (21.15%), (E)-Tagetone (16.21%), (Z)-Tagetone (14.99%), (Z)-beta-Ocimene (9.84%), Limonene (7.40%), and allo-Ocimene (6.69%) represent more than 70% of the essential oil. The results of this study are consistent with those found by Farshbaf Moghaddam *et al.* [61] and Garcia *et al*. [62]. The *T. minuta* essential oil used in this study was rich in terpenes, as determined by GC and GC–MS analyses.

The bioassay results of this study demonstrate that both *T. minuta* and *C. citratus* are highly potent against *P. duboscqi* sandflies. Between the two oils tested, that of *C. citratus* was significantly more potent ($P < 0.05$) and caused higher mortality than that of *T. minuta* on both against male and female sandflies. The results further demonstrate that after 24 h, treatment with the oil of *C. citratus* at a concentration of 1 mg/ml caused mortality of 91.11 and 88.89 % against female and male sandflies, respectively while *T. minuta* oil at the same concentration, recorded a relatively lower mortality of 71.11% 66.67 % in female and male sand flies, respectively. The results of this study demonstrate that, the effects of the oils were dose-dependent and increased with the concentration of the oil. The low concentrations tested inflicted low levels of mortality. The highest mortality levels were observed at 72 h after treatment for all the concentrations tested. In fact, after 72 h after treatment, the essential oils of *C. citratus* and *T. minuta* at a concentration of 1 mg/ml recorded a mortality of 100.00 and 82.22 % respectively, on female sandflies. At the same concentration, *C. citratus* and *T. minuta* oils caused mortalities of 100.00 and 88.89% respectively, in male sandflies.

The findings of this study concur with previous studies which demonstrated that *C. citratus* and *T. minuta* essential oils are effective against arthropods. Hanifah *et al*. [63] was able to demonstrate that the mortalities from lemongrass extract were higher than neem for both topical and contact activities against the house dust mites *Dermatophagoides farinae* (*D. farinae*) and *Dermatophagoides pteronyssinus* (*D. pteronyssinus*). At 50 % concentration, both 24 hrs topical and contact exposures to lemon grass resulted in more than 91% mortalities for both species of mites. At the same concentration and exposure time, neem resulted in topical mortalities of 40.3% and 15.7% against *D. pteronyssinus* and *D. farinae* respectively; contact mortalities were 8.0% and 8.9% against the 2 mites, respectively [63].

Previous studies have demonstrated various biocidal activities of plant natural oils and products against sandfly adults. *Lutzomyia longipalpis* Lutz & Neiva adults were killed by water extracts of the leaves of *Antonia ovata* Pohl (LD 50 =233mg/mL) and water extracts of the roots of *Derris amazonica* Killip (LD 50 =212mg/ mL) [64]. Also, *Eucalyptus* spp. essential oils exhibit toxic effects in contact with *L. longipalpis* adults. Thus, adulticidal effects were observed for lemon ironbark (*E. staigeriana* F. Muell) essential oil whose major components were limonene, Z-citral, α –citral (EC50 = 0.59mg/ml), and lemon eucalyptus (*E. citriodora* Hook) with the major chemical constituent being β-citronellal (ED50= 5.04mg/ml). Finally, *E. globulus* Labill with essential oil major component being 1,8-cin-eole with an effective concentration of 7.78mg/ml. The superior toxicity of lemon ironbark is evident from these and other data and is due presumably due to the activity of the major components of its essential oil, which were not individually evaluated for biological activity [65].

Hanifah *et al.* [63] reported that mortalities from lemongrass extract were higher than neem for both topical and contact activities. At 50 % concentration, both 24 hrs topical and contact exposures to lemongrass resulted in more than 91% mortalities for both species of mites. At the same concentration and exposure time, neem resulted in topical mortalities of 40.3% and 15.7% against *D. pteronyssinus* and *D. farinae* respectively; contact mortalities were 8.0% and 8.9% against the 2 mites, respectively. There was no

difference in topical mortalities of *D. pteronyssinus* from exposure to concentrations of lemongrass and neem up to 12.50%; lemongrass was more effective than neem at the higher concentrations [63].

I conclude that the two essential oils are promising natural repellents due to their safety advantage over chemical repellents. I therefore, recommend further studies on human subjects in the field before their adoption for use against Phlebotomine sandflies.

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6. Conflict of Interest

The author declares that there is no conflict of interest in the publication of this work.

7. References

1. Killick-Kendrick R. Phlebotomine vectors of the leishmaniases:a review. Med. Vet. Entomol. 1990; 4:1-24.
2. WHO Expert Committee. Control of the leishmaniasis: report of a meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva, 22–26 March 2010. WHO. Tech. Rep. Ser. 2010; 949:1-186.
3. Seccombe AK, Ready PD, Huddleston LM. A catalogue of Old World phlebotomine sandflies (Diptera: Psychodidae, Phlebotominae). Occ. Pap. Syst. Entomol. 1993; 8:1-57.
4. Young DG, Duncan MA. Guide to the Identification and Geographic Distribution of Lutzomyia Sand Flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). Gainesville, FL: Assoc. Publ. Mem. Am. Entomol. Inst. 1994; 881 pp.
5. Alexander B, Maroli M. Control of Phlebotomine sandflies. *Med. Vet. Entomol.* 2003; 17:1-18.
6. Tesh RB. The genus *Phlebovirus* and its vectors. *Annu. Rev. Entomol*. 1988; 33:169-81.
7. Hambuch TM, Handley SA, Ellis B, Chamberlin J, Romero S, Regnery R. Population genetic analysis of *Bartonella bacilliformis* isolates from areas of Peru where Carrion's disease is endemic and epidemic. J. Clin. Microbiol. 2014; 42:3675-80.
8. Cohnstaedt LW, Beati L, Caceres AG, Ferro C, Munstermann LE. Phylogenetics of the phlebotomine sand fly group Verrucarum (Diptera: Psychodidae: Lutzomyia). Am. J. Trop. Med. Hyg. 2011; 84:913-22.
9. World Health Organization, leishmaniasis burden home page:http://www.who.int/leishmaniasis/burden/en. Accessed on 26th June 2015.
10. Heisch RB, Wijers DJ, Minter DM. In pursuit of the vector of Kala-azar in Kenya. Br. Med. J. 1962; 1:1456-1458.
11. Tonui WK. Situational analysis of leishmaniasis research in Kenya. Afr. J. Health. Sci. 2006; 13:7-21.
12. Beach R, Kiilu G, Hendricks L, Oster C, Leeuwenburg J. Cutaneous leishmaniasis in Kenya. Transmission of *Leishmania major* to man by the bite of a naturally infected *Phlebotomus duboscqi.* Trans. R. Soc. Trop. Med. Hyg. 1984; 78:747-751.
13. Davies CR, Kaye P, Croft SL, Sundar S. Leishmaniasis: new approaches to disease control. BMJ 2003; 326:377-382.
14. Chappuis F, Sundar S, Hailu A, Ghalib H, Rijal S, Peeling RW, *et al*. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nat Rev Microbiol. 2007; 5:873-82.
15. Gontijo CMF, Melo MN. Leishmaniose Visceral no Brasil: quadroatual, desafios e perspectives. Rev Bras Epidemiol. 2004; 7:338-349.
16. Viegas-Júnior C. Terpenos com Atividade inseticida: uma alter-nativapara o control equímico de insetos. Quim Nova. 2003; 26:390-400.
17. De Paula JP, Farago PV, Checchia LEM, Hirose KM, Ribas JLC. Atividaderepelente do o´ leoessencial de Ocimumselloi Benth (variedadeeugenol) contra o *Anopheles braziliensis* Chagas. Acta Farm Bonaerense. 2004; 23:376-378.
18. Regnault-Roger C, Vincent C, Arnason JT. Essential Oils in Insect Control:Low-Risk Products in a High-Stakes World. Annu Rev Entomol. 2012; 57:405-24.
19. Keita SM, Vincent C, Schmit JP, B´elanger A. Essential oil composition of *Ocimum basilicum* L., *O. gratissimum* L. and *O. suave* L. in the Republic of Guinea. Flavour Fragr J. 2000; 15:339-41.
20. Adorjan B, Buchbauer G. Biological properties of essential oils:an updated review. Flavour Fragr J. 2010; 25:407-426.
21. Nerio LS, Olivero-Verbel J, Stashenko E. Repellent activity of essential oils:a review. Bioresour Technol. 2010; 101:372-8.
22. Olivero-Verbel J, Nerio LS, Stashenko EE. Bioactivity against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) of *Cymbopogon citratus and Eucalyptus citriodora* essential oils grown in Colombia. Pest Manag Sci. 2010; 66:664-668.
23. Ansari MA, Razdan RK. Repellent action of *Cymbopogon martini martini* Stapf. var. Sofia against mosquitoes. Indian J Malariol. 1994; 31:95-102.
24. Tawatsin A, Wratten SD, Scott RR, Thavara U, Techadamrongsin Y. Repellency of volatile oils from plants against three mosquito vectors. J Vector Ecol. 2001; 26:76-82.
25. Shah G, Shri R, Panchal V, Sharma N, Singh B, Mann AS. Scientific basis for the therapeutic use of *Cymbopogon citratus*, stapf (Lemon grass). J Adv Pharm Technol Res. 2011; 2:3-8.
26. Arias RJ, Schmeda-Hirschmann G, Falcao A. Phytother. Res, 1992; 6:64-67.
27. Aziz EE, Abbas MH. Chemical composition and efficiency of five essential oils against the Pulse beetle Callosobruchus maculates F. on Vignaradiata seeds. Am. Eurasian J Agric Environ Sci, 2010; 8:411-419.
28. Kabera J, Gasogo A, Uwamariya A, Ugirinshuti V, Nyetera P. Insecticidal effects of essential oils of *Pelargonium raveolens and Cymbopogon citratus* on *Sitophilus zeamais* (Motsch). Afr J Food Sci. 2011; 5:366-375.
29. Phasomkusolsil S, Soonwera M. Efficacy of herbal essential oils as insecticide against *Aedes aegypti* (Linn.), *Culex quinquefasciatus* (Say) and *Anopheles dirus*(Peyton and Harrison). Southeast. Asian J Trop Med Public Health. 2011; 42:1083-1092.
30. Pushpanathan T, Jebanesan A, Govindarajan M. Larvicidal, ovicidal and repellent activities of *Cymbopogan citratus* Stapf (Graminae) essential oil against the filarial mosquito *Culex quinquefasciatus* (Say) (Diptera: Culicidae). Trop. Biomed 2006; 23:208-

212.
31. Hindumathy CK. *In vitro* Study of Antibacterial Activity of *Cymbopogon Citratus. World Academy of Science, Engineering and Technology*, 2011; 74:193-197.
32. Matasyoh JC, Wagara IN, Nakavuma JL, Kiburai AM. Chemical composition of *Cymbopogon citratus* essential oil and its effect on mycotoxigenic *Aspergillus* species. Afr. J. Food. Sci. 2011; 5:138-142.
33. Syed M, Qamar S, Riaz M, Chaudhary FM. Pak J Sci Ind Res. 1995; 38:146-148.
34. Akin-Osanaiye BC, Agbaji AS, Dakare MA. Antimicrobial activity of oils and extracts of *Cymbopogon citratus* (Lemon Grass), *Eucalyptus citriodora* and *Eucalyptus camaldulensis*. J Med Sci. 2007; 7:694-697.
35. Maia MF, Moore SJ. Plant-based insect repellents:a review of their efficacy, development and testing. Malar J. 2011; 4:1-10.
36. Green MM, Singer JM, Sutherland DJ, Hibben CR. Larvicidal activity of *Tagetes minuta* (marigold) toward *Aedes aegypti*. J Am Mosq Control Assoc 1991; 7:282-286.
37. Perich MJ, Wells C, Tredway KE. Toxicity of extracts from tree *Tagetes* against adult and larvae of yellow fever mosquito and *Anopheles stephensi* (Diptera:Culicidae). J Med Entomol. 1994; 31:833-837.
38. Macedo ME, Consoli RA, Grandi TS, dos Anjos AM, de Oliveira AB, Mendes NM, *et al*. Screening of Asteraceae (Compositae) plant extracts for larvicidal activity against *Aedes fluviatilis* (Diptera: Culicidae). Mem Inst Oswaldo Cruz. 1997; 92:565-70.
39. Ireri LN, Kongoro J, Ngure P, Mutai C, Langat B, Tonui W, *et al*. The potential of the extracts of *Tagetes minuta* Linnaeus (Asteraceae), *Acalypha fruticosa* Forssk (Euphorbiaceae) and *Tarchonanthus camphoratus* L. (Compositae) against *Phlebotomus duboscqi* Neveu Lemaire (Diptera: Psychodidae), the vector for *Leishmania major,* Yakimoff and Schokhor. J Vector Borne Dis. 2010; 3:168-74.
40. Mong'are S, Ng'ang'a Z, Maranga R, Osiemo Z, Ngure P, Ngumbi P, *et al*. Effect of Leaf Crude Extracts of *Tarchonanthus Camphoratus* (Asteraceae), *Acalypha Fruticosa* (Fabacea) and *Tagetes Minuta* (Asteraceae) on Fecundity of *Phlebotomus Duboscqi*. American Int J Cont Res. 2012; 2:194-200.
41. Adeniran OI, Fabiyi E. A cream formulation of an effective mosquito repellent:a topical product from lemongrass oil (*Cymbopogon citratus*) Stapf**.** J Nat Prod Plant Resour. 2012; 2:322-327.
42. Clevenger JF. Apparatus for the determination of volatile oil. J Am Pharm Assoc. 1928; 17:345-349.
43. Evans WC. Trease and Evans' Pharmacognosy. Thirteenth edition. *Oxford University Press*, London, 1989.
44. Nchu F, Magano SR, Eloff JN. In vitro anti-tick properties of the essential oil of Tagetes minuta L., (Asteraceae) on Hyalomma rufipes (Acari:Ixodidae). Onderstepoort J Vet Res. 2012; 79:E1-5.
45. Mburu DM, Ochola L, Maniania NK, Njagi PGN, Gitonga LM, Ndung'u MW, *et al*. Relationship between virulence and repellency of *Metarhizium anisopliae* and *Beauveria bassiana* towards *Macrootermes michaelseni* and chemical identification of the mediating signals. J Ins Phy. 2009; 55:774-780.
46. Adams RP. Identification of essential oil components by Gas Chromatography/ Mass spectroscopy. Carol Stream, USA. Allured Publishing Corp, 1995, 46-449.
47. Beach R, Young DG, Kiilu G. New Phlebotomine sandfly colonies II. Laboratory colonization of *Phlebotomus duboscqi* (Diptera: Psychodidae). J Med Entomol. 1986; 23:114-115.
48. Abbot WS. A method of computing the effectiveness of an insecticide. J Econ Entomol. 1925; 18:265-267.
49. Finney DJ. *Probit analysis*, III edn. London: Cambridge University Press, 1971; p. 1-333.
50. Chisowa EH, Hall DR, Farman DI. Volatile constituents of the essential oil of *Cymbopogon citratus* Stapf grown in Zambia. Flavour Frag J. 1998; 13:9-30.
51. Kasali AA, Oyedeji AO, Ashilokun AO. Volatile leaf oil constituents of *Cymbopogon citratus* (DC) Stapf. Flavour Frag J. 2001; 16:377-378.
52. Menut C. Larvicidal activity against *Anopheles gambiae* Giles and chemical composition of essential oils from four plants cultivated in Cameroon. Biotechnol. Agron. Soc. Environ. 2009; 13:77-84.
53. Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, *et al*. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry, 2005; 91:621-632.
54. Sidibe L, Chalchat JC, Garry RP. Aromatic plants of Mali (IV):Chemical composition of essential oils of *Cymbopogon citratus* (DC) Stapf and *C. giganfeus* (Hochst) Chiov. J Essent Oil Res. 2001; 13:110-2.
55. Boukhatem MN, Ferhat MA, Kameli A, Saidi F, Kebir HT. Lemon grass (*Cymbopogon citratus*) essential oil as a potent anti-inflammatory and antifungal drugs**.** Libyan J Med. 2014; 9:25431
56. Vázquez-Briones MC, Hernández LR, José Á, Guerrero-Beltrán JÁ. Physicochemical and Antioxidant Properties of *Cymbopogon citratus* Essential Oil. J Food Res. 2015; 4(3).
57. Ekpenyong CE, Akpan EE, Daniel NE. Phytochemical Constituents, Therapeutic Applications and Toxicological Profile of *Cymbopogon citratus* Stapf (DC) Leaf Extract**.** J Pharmacognosy and Phytochem Res. 2014; 3:133.
58. Farhang V, Amini J, Javadi T, Nazemi J, Ebadollahi A. Chemical composition and antifungal activity of essential oil of *Cymbopogon citratus* (DC.) Stapf. against three *Phytophthora* Species. Greener J Bio Sci. 2013; 3:292-298.
59. Gupta A, Sharma S, Naik SN. Biopesticidal value of selected essential oils against pathogenic fungus, termites, and nematodes. Int Biodeter Biodeg. 2011; 65:703-707.
60. Rahimi-Nasrabadi M, Nazarian S, Farahani H, Fallah-Koohbijari GR, Ahmadi F, Batooli H. Chemical Composition, Antioxidant, and Antibacterial Activities of the Essential Oil and Methanol Extracts of *Eucalyptus largiflorens* F. Muell. Int. J. Food. Proper. 2013; 16:369-381.
61. Farshbaf M, Moghaddam M, Omidbeigi R, Sefidkon F. Chemical composition of essential oil *Tagetes minuta* from Iran. Iran J pharm Res. 2004; 3:83-84.
62. Garcia MV, Matias J, Barros JC, de Lima DP, Lopes Rda S, Andreotti R. Chemical identification of *Tagetes minuta* Linnaeus (Asteraceae) essential oil and its acaricidal effect on ticks. Rev Bras Parasitol Vet. 2012; 21:405-11.
63. Hanifah AL, Awang SH, Ming HT, Abidin SZ, Omar MH. Acaricidal activity of *Cymbopogon citratus* and

Azadirachta indica against house dust mites. Asian Pac J Trop Biomed. 2011; 365-369.
64. Luitgards-Moura JF, Castellon BEG, Rocha AF, Tsouris P, Rosa-Freitas MG. Preliminary assays indicate that *Antonia ovata* (Loganiaceae) and *Derris amazonica* (Papilionaceae), ichthyotoxic plants used for fishing in Roraima, Brazil, have an insecticide effect on *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae). Mem Inst Oswaldo Cruz. 2002; 97:737-742.
65. Maciel MV, Morais SM, Bevilaqua CML, Silva RA, Barros RS, Sousa RN, Sousa LC, *et al*. Chemical composition of Eucalyptus spp. essential oils and their insecticidal effects on *Lutzomyia longipalpis*. Vet Parasitol. 2010; 167:1-7.