REPELLENCYEFFECTS OF *Pelargonium citrosum*AND *Rosmarinusofficinalis*ESSENTIAL OILSAGAINST HOUSEFLY, *Musca domestica*L. (DIPTERA: MUSCIDAE)

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ABSTRACT

Use of botanical environmentally friendly and biodegradable insect repellants as opposed to chemical insecticides is increasingly becoming important as an alternative method of insect control. Housefly (*Musca domestica* L.) haspotential of transmitting pathogen causing diseases such as typhoid, cholera, bacillary dysentery, tuberculosis, anthrax, ophthalmia, and parasitic worms.The aim of this study was to evaluate the repellency of oil extracted from the leaves of *Pelargonium citrosum* and *Rosmarinus officinalis*. Extraction of essential oils was by hydro-distillation. Condensed oilextracts were collected in n-hexane and insect behavioral response tested using adult houseflies (*Musca domestica* L.). *N, N*-diethyl-*m*-toluamide (DEET) was used as the positive control and acetone as the negative control. The bioactive oil was then analyzed using GC-MS. The characteristic volatiles obtained from the two ~~oils~~ plantsshowed different compositions. *P. citrosum*oil comprised mainly ~~of Linalool~~of Linalool, Geraniol, m-Camphorene,2-naphthalenemethanol-1,2,3,4,4a,5,6,7-octahydroalpha, Geranylangelatewhile*R.officinalis* comprised mainly ofα-Pinene, , Eucalyptol,α-Terpinenol. Dose-response evaluations of these oils showed that *R. officinalis*oil was more repellent (LD_{50} = 0.299 mg) than that of *P. citrosum*(LD_{50} = 0.445 mg). The results provide scientific rationale for traditional use of*R. officinalis*~~ oil~~ and*Pelargonium* ~~*citrosum*~~*citrosum*essential oilsin control ofhousefly and other common insects in the household.

Key words: Essential oils, GC-MS, LD_{50},LD_{75}, *Musca domestica* L,*P. citrosum and R. officinalis*

INTRODUCTION

Environmental pollution because of continued use of conventional pesticides is increasing as population increases in developing countries (Pavela,2008). Botanical pesticides are better alternatives as they are environmentally friendly and biodegradable as opposed to chemical insecticides. They have repellent, insecticidal, antifeedant and insect growth regulator effects (Hashem and Youssef, 1991).

Musca domestica L. is a major problem to man in developing towns and industries (Axtell and Arends, 1990). Housefly has potential of transmitting more than a hundred pathogen causing diseases to human and animals such as typhoid, cholera, bacillary dysentery, tuberculosis, anthrax, ophthalmia, and parasitic worms (Wasa, *et al*., 1999, Olsen *et al*., 2001). The fly transmits pathogenic organism from garbage, sewage, latrine and other sources of filth. They carry by mouth part through vomiting, feaces, and external body parts to foods (Zurek *et al*., 2001).

*Pelargonium citrosum*isa perennial sub-shrub with fragrant leaves that are reminiscent citronella. It is marketed as mosquito plant in the United States and Canada. Chemical analysis by the authors revealed that combined essential oils of fresh greenhouse and field grown citrosa have, 6.2% geraniol, 10.4% citronellol, 8.9% isomenthone and 6.8% linalool.The effectiveness of the citrosa as a repellent against field populations of spring *Aedes*spp mosquitoes was evaluated and compared with a 75% DEET (N, N-diethyl-*m*-toluamide)formulation. DEET provided >90% reduction in mosquitoes, biting subjects for up to 8 hours post treatment.

Rosmarinus officinalis commonly known as Rosemary is a woody perennial herb with fragrant evergreen, needle-like leaves and white, pink, purple or blue flowers native to the Mediterranean region and Asia. It is a member of the mint family (lamiaceal) which includes many other herbs. ~~It forms range from upright to trailing. The upright forms can reach 1.5m (5ft) tall.~~ Upon cultivation the leaves, twigs and flowering apices are extracted for use. Rosemary is used as a decorative plant in gardens where it may have pest control effects. The leaves are used to flavor various foods such as stubbing and roast meat.

Rosemary contains a number of phytochemicals including rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, carnosic acid and carnosol (vallverdu-Queralt*et al.,*2014). In traditional medicine extracts and essentials oil from flowers and leaves are used in the belief they may be used to treat a variety of disorders. (vallverdu-Queralt *et al*., 2013).In the middle age R~~r~~osemary was associated with wedding ceremony then from this association, R~~r~~osemary was thought to be a loved charm. It has long had a popular reputation for improving memory. The Guardian paper reported in 2017 that sales of rosemary's oil to students in the UK studying for examinations had skyrocketed because of rosemary's perceived benefits to memory (Emine Saner, 2017).

MATERIALS AND METHODS

Sample collection and extraction

The leaves of *Pelargonium citrosum* and *Rosmarinus officinalis* were collected from Githunguri in Kiambu County of Kenya, based on the~~ir~~ reported ethnobotanical information as an insect repellent~~s~~. The fresh leaves were washed with running tap water and cut into small pieces, air dried at room temperature then pulverized with an in-house mechanical blender. Extraction of essential oils from the ground plant samples were done through hydro-distillation using Clevenger apparatus. The condensed extract was collected in n-hexane and filtered using Whatman® grade 1 filter paper containing anhydrous Sodium Sulphate to remove any traces of water. Hexane was then removed by distillation at 60^{o}C using 'Contes' Short Path distillation, and the collected oil weighed into smaller amber covered vials and stored at 4^{o}Cready for repellency tests. The percentage yield of the extracts was; 7.28% for *R. officinalis* and 10.43% for *P. citrosum*

GC-MS analysis of the repellent oil

The bioactive oil was analyzed using gas chromatography coupled with a M~~m~~ass S~~s~~pectrometer, (GC-MS~~).The~~). The oil sample was prepared in dichloromethane, and an aliquot of 0.5 µL injected into GC-MS under the following conditions: Temperature ramp: 60^{o}C (2 min); at 8^{o}C/min to 250^{o}C (3 min); Column: DB-XLB (standard non-polar); Injection mode: Split, 200:1; Mass range: 40 – 470 u; Source temp: 200^{o}C; Interface temp: 250^{o}C; Carrier gas: He, 99.999% purity; Flow rate: 1 ml/min; and Pressure of 8kPa.

Repellency bioassays

Short term repellency bioassays

Insect behavioral response to the repellent test oil was determined using adult houseflies (*Musca domestica* L.). This provides a measure of contact irritancy that is more applicable for use in the control of structure-invading pests. Test solutions of various concentrations of extract oil and *N, N*-diethyl-*m*-toluamide (DEET) were prepared consisting of 5.0,3.0, 2.0, 1.0, 0.5, 0.3, 0.2, 0.1, 0.05 and 0.025% (v~~o~~l/v) in acetone (Schultz *et al*., 2004). DEET was used as the positive control and acetone as the negative control.

One milliliter of test solution or solvent was applied to one-half of 12.5-cm-diameter round filter paper with an area of 61 cm^2 and allowed to dry. A solvent-only half-piece of filter paper (control) was placed in the remaining one-half of the 15-cm plastic petri dish after solvent evaporation. At the time of initiation, a single insect was introduced through a centered hole in the petri dish lid and covered with masking tape. The time the insect spent on treated and control filter paper out of 5 min (300 s) was recorded using two stopwatches. Ten replicates~~ions~~ for each concentration were performed, and the percentage repellency calculated using the formula: [(Time on Untreated – Time on Treated)/300] × 100%.

Extended Repellency Bioassay

Test solutions were made consisting of 5,3, 2, 1, 0.5, 0.3, 0.2, 0.1, 0.05 and 0.025% concentrations by volume in acetone for the steam distillate oil and DEET. One-half of a 12.5-cm filter paper with an area of 61 cm^2 was treated with 1ml of test solution and allowed to dry (30s) before being placed in a 15-cm petri dish. The other half of the filter paper was treated only with solvent (acetone) for control. One adult housefly was placed in each petri dish and enclosed by a mesh, to eliminate any fumigation effects and allow volatilization of the repellent under ambient laboratory conditions. The location of the insect (presence on the treated or untreated filter paper) was recorded at seven time-points after initiation: 15 min, 30 min, 1 h, 2h, 4h, 6h, and 24h. Ten replicates were done for each specific preparation.

Data Analysis

Dose-response data were subjected to simple regression and probit analysis using the percent repellency values obtained from replicated experiments and a regression model developed based on the equation below;

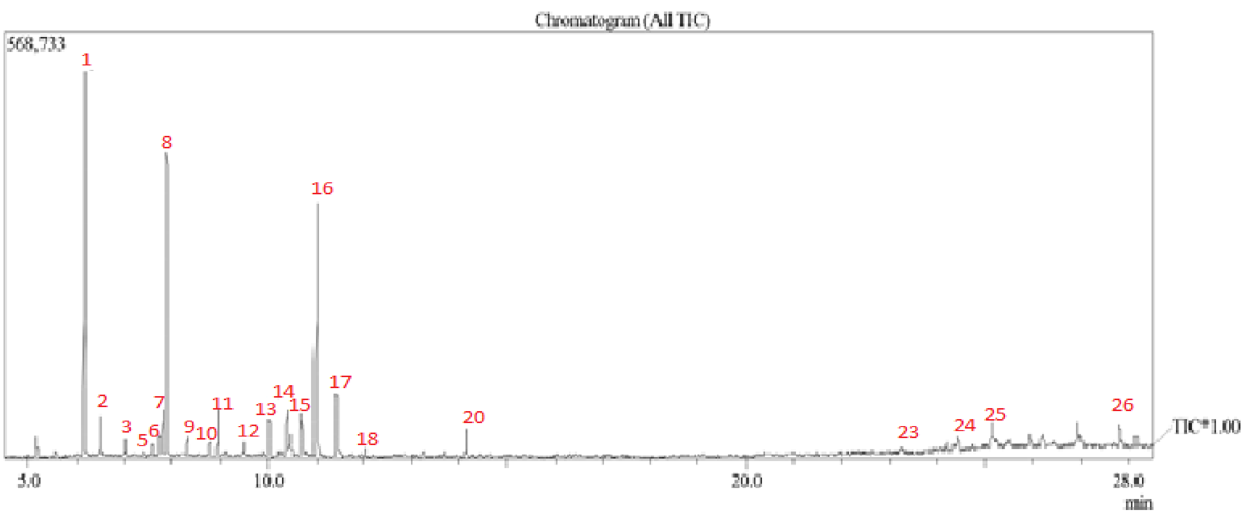
$$\text{Probit}[\Pi(\text{dose } 1)] = \beta_0 + \beta_1 x + \epsilon,$$

Where β_0 is the coefficient of the model representing *y*-intercept;, β_1 is the coefficient of the model representing dose1;, *x* is the various concentrations of essential oils;, dose1 is the Log_{10} (dose);, ϵ is the error term (residual term) representing the difference between the actual observed value and that predicted by the model variable;, *x* is the dose of the essential oil and Π is the repellency probability.

RESULTS AND DISCUSSION

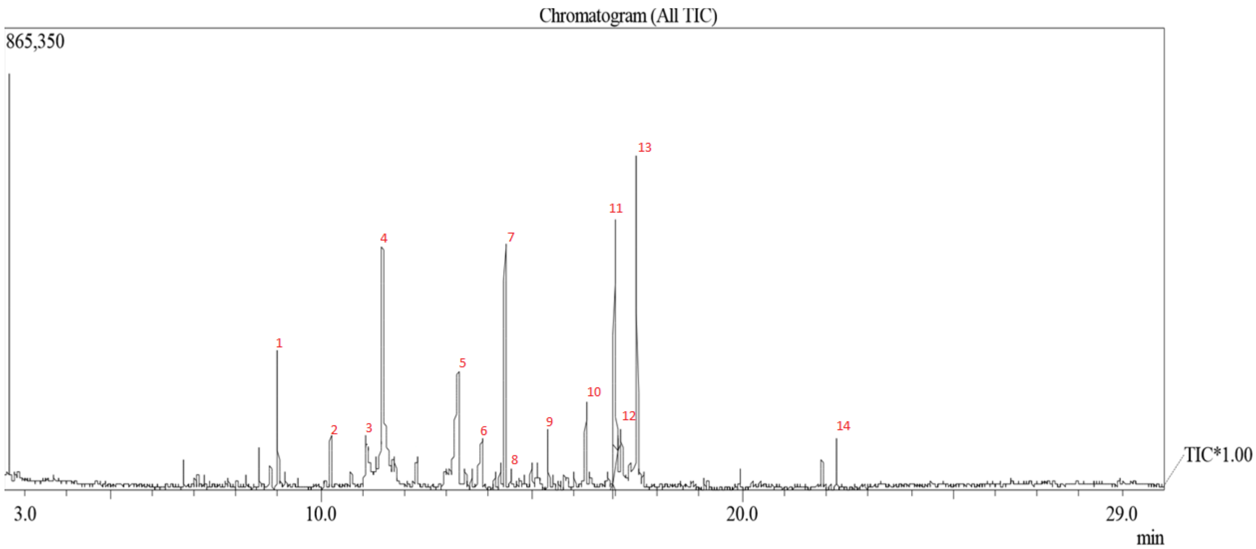
Figure 1 below shows the GC-MS chromatogram of oils extracted from *R.offinalis* and figure 2 the chromatogram of oils extracted from *P. citrosium*.

Fig 1. GC-MS profiles of essential oils from *R. officinalis*



1=α-Pinene, 2=Camphene, 3=β-pinene, 4=β-myrcene, 5=α-terpinene, 6=β-cymene, 7= D-Limonene, 8=Eucalyptol, 9=γ-terpiinene 10= Isoterpinolene, 11=Linalools, 12=2-Pinen-7-one, 13=Camphor, 14=Borneol, 15=Terpinen-4-ol, 16=α-Terpinenol, 17=2-Pinen-4-one, 18=Cis-Geraniol, 19=2-Camphanol acetate, 20=Geraniol acetate, 23=Ferruginol, 24=Isocarnosol, 25=α-pentyl-4-oxa-~~5,beta~~5, beta-androstane3-17-dione, 26=Demethylsalvicanol.

Fig.2GC-MS profiles of essential oils from*P. citrosum*



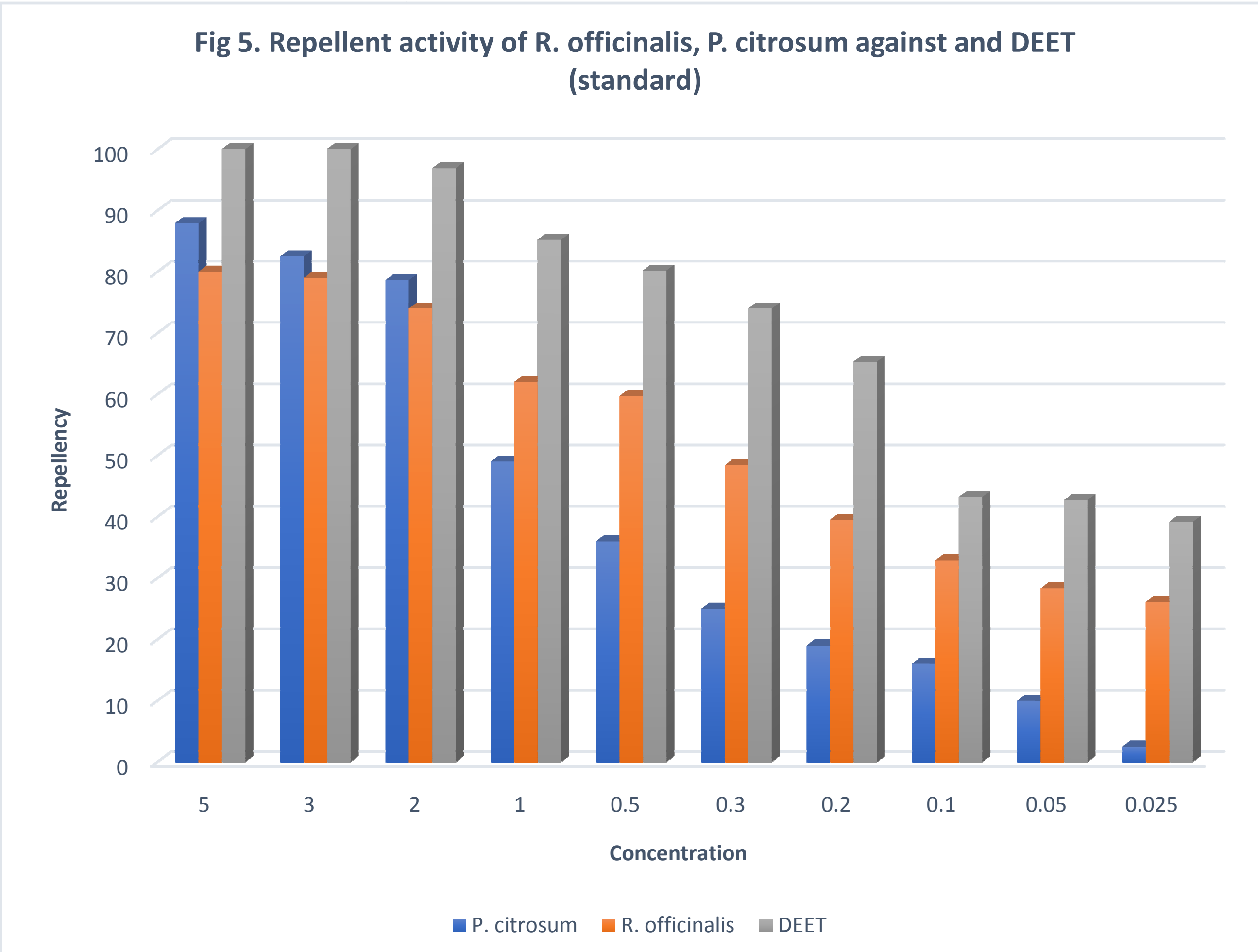
1=Linalool, 2=2-isopropyl-5-5methylcyclohexanone(p-menthone), 3=Trans-farnesol, 4=Geraniol, 5=Decanoic acid, 6=8-methyl-6-nonenoic acid, 7=m-Camphorene, 8=1S,2R,5S-Guia-6,9-diene, 9=Delta-cadinene, 10=2-phenylethyltiglate, 11=2-naphthalenemethanol-1,2,3,4,4a,5,6,7-octahydroalpha, 12=5-Azulenemethanol, 13=Geranylangelate, 14=Geranylacetate.

GC-MS analysis of *R. officinalis* revealed compounds toxic to pests and parasites. α-pinene, Eucalyptol, α-terpinenol, 2-pinen-4-one and Linalool, for instance, found in *R. officinalis* are toxic to eggs and larvae of insects (Liu *et al*., 2011). Linalool, Geraniol, capric acid, m-camphorene, delta-cadinene, 2-phenylethyltiglate and Geranylangelate found in *P. citrosum* have previously~~s~~demonstrated repellency against arthropod pests (Tapondjou *et al*., 2005).

Essential oils interfere with basic metabolic biochemical, physiological and behavioral functions of arthropods. They inhale, ingest or absorb essential oils. The rapid actions against some pests is indicative of a neurotoxic mode of action and there is evidence for interference with the neuromodulator octopamine (Enan.,2005) or GABA-gated chloride channels (Priestley *et al*., 2003; Khater 2011).

Repellent activity

Fig3. Below shows the correlation between the repellent activities of the plant extracts and the standard



Probit analysis results

Table 4: Lethal dose at 50% and at 75% concentration.

Sample	LD_{50} (mg)	LD_{75} (mg)
R. officinalis	0.299	2.487
P. citrosum	0.445	1.820

Key: LD_{50}-lethal dose at 50% repellency; LD_{75}-lethal dose at 75% repellency.

The repellence of the two essential oils at different doses is shown in figure 5 above. The essential oil of *R. officinalis* was found to be significantly more effective in insect repellency than that of *P. citrosum*at low corresponding doses, however *P. citrosum*showed more repellency against the housefly at higher doses. In both the essential oils of *P. citrosum*and *R. officinalis,* there was significant correlation between repellence and dose (Pearson Correlation, $\alpha = 0.01$).

Comprehensive repellence studies with *R. officinalis* and*P. citrosum*oils at ten doses confirmed the higher repellence of the former against *Musca domestica L.* Interestingly, its repellent effect is comparable to that of commonly used repellent DEET at 0.1 mg dose (with essential oil of *R. officinalis* producing a repellent effect of 32.9 ± 1.3% compared with that of DEET, 43.2 ± 5.8%)).

Relative to controls, 2% DEET provide > 90% repellency after 8 hours and>70% repellency for the two study plant extracts.

Probit analysis results

Table 14: Lethal dose at 50% and at 75% concentration.

Sample	LD_{50} (mg)	LD_{75} (mg)
R. officinalis	0.299	2.487
P. citrosum	0.445	1.820

Key: LD_{50}-lethal dose at 50% repellency; LD_{75}-lethal dose at 75% repellency.

Model development of the bioassay data of the two essential oils allowed estimation of LD_{50} and LD_{75} (Table 4), above.*R. officinalis*oil was found to be more effective at LD_{50}compared to *P. citrosum*.,Tthis was however not the case at LD_{75}.*P. citrosum*oil exhibited higher repellency at a higher concentration (LC_{75}). The high repellency of *R. officinalis*oil at lower concentration can be attributed to the easy mobility of the 26 compounds leading to more evaporation hence effective repellency. Higher concentrations of *R. officinalis*oil lead to more hydrogen bonding between the compounds hence reducing their ability to vaporize.

Previous work has been done on Thyme oil having repellent action on varroa mite (Natalia *et al*., 2009). Larvicidal efficiency determined for thymol was lowest doses LD_{50}32.9 and 14.2mg/L for the third and fourth instars of *Culex quinquefasciatus*. According to (Roman, 2009),Ceclover leaf oil showed good repellency at a concentration of 0.005mg/cm^2 against *Culex pipiens.* In this way a number of essential oils showed insecticidal properties against different insect pest so that there is an increasing interest in developing plant origin insecticides.

As can be concluded from the data presented on plant volatiles, each species seems to have its own unique chemical composition with little similarity. In summary, the results of this study,further strengthens the view that *R. officinalis* and *P. citrosum* are potential sources of insect repellants. The results provide scientific rationale for traditional use of raw products of these plants in controlling housefly, ***Musca domestica*L. (DIPTERA: MUSCIDAE)**

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