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ESSENTIAL OIL ANALYSIS AND FIELD EVALUATION OF THE CITROSA PLANT "*PELARGONIUM CITROSUM*" AS A REPELLENT AGAINST POPULATIONS OF *AEDES* MOSQUITOES

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ABSTRACT. A plant recently introduced into North America as the citrosa, *Pelargonium citrosum* ('Van Leenii'), has been marketed as a biological repellent against mosquitoes. Citrosa is claimed to repel mosquitoes within a 10 ft.^{2} (0.93 m^2) area due to a continuous fragrant release of citronella oil. The total essential oil yield was 0.2 ± 0.1% from fresh plant material. Chemical analysis by the authors revealed that combined essential oils of fresh greenhouse- and field-grown citrosa have 35.4 ± 6.2% geraniol, 10.4 ± 1.6% citronellol, 8.9 ± 2.0% isomenthone, and 6.8 ± 3.8% linalool. Both the morphology and essential oil of citrosa fall within the *Pelargonium* × *asperum* hybrid complex and are similar to 'Rosé', the commercial rose geranium. No character of morphology or essential oil of a *Cymbopogon* species yielding commercial citronella oil could be detected in the citrosa. 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-3-methylbenzamide) formulation. Deet provided >90% reduction in mosquitoes biting subjects for up to 8 h post-treatment. There was no significant difference between citrosa-treated and nontreated subjects.

INTRODUCTION

A new biological repellent against mosquitoes has generated considerable interest in North America. This new agent is the citrosa plant and has been marketed as *Pelargonium citrosum* ('Van Leenii'). The promotional literature for citrosa claims that it was genetically engineered to effectively repel mosquitoes indoors and outdoors by the continuous release of a mosquito-repelling fragrance.[3] Other promotional literature states that this plant was created in Holland by implantation of genes that produce citronella oil from a Chinese grass of citronella into a plant of the genus *Pelargonium* in the Geraniaceae family.[4] One citrosa plant is reputed to repel mosquitoes within a 10 ft.^{2} (0.93 m^2) area.[5] Morphologically, the citrosa plant is similar to *Pelargonium* 'Rosé' (*P. graveolens* Auct.), or rose geranium, and produces a similar odor. To determine the validity of claims made by producers and vendors of the citrosa plant, chemical analysis was conducted on the citrosa to identify essential oils in common with Chinese grass of citronella.

Preliminary laboratory experiments in which exposed hands were placed in cages containing *Aedes aegypti* (Linn.) adults indicated that the citrosa plant provided no protection when present in a cage and minimal protection if rubbed on the hand. This prompted the initiation of a field trial to test the effectiveness of the citrosa plant as a repellent against spring *Aedes* spp., which constitute the major pest species that bite people in southwestern Ontario. Recent work has shown that the citrosa plant does not protect human subjects from biting *Aedes albopictus* (Skuse) and *Culex quinquefasciatus* Say in Florida (Cilek and Schreiber 1994) or various spring *Aedes* spp. and *Coquillettidia perturbans* in Michigan (Cummings and Craig 1995).

MATERIALS AND METHODS

For essential oil analysis, 6 citrosa plants were grown in a greenhouse by a licensed distributor in Maryland and harvested in November 1991, and 3 citrosa plants were grown outdoors on Sassafras sandy loam (pH 6.6) in Camden, DE, and harvested in September 1992. To assess the variation of the essential oil of citrosa with respect to the *Pelargonium* × *asperum* complex, 3 replicates of 'Rosé' were greenhouse-grown by a commercial grower in Illinois and harvested in February 1992. Voucher specimens were filed at the Claude E. Phillips Herbarium, Delaware State University, Dover. The terminal 0.5–1 m of leaves and stems was subjected to steam distillation, gas chromatography, and mass spectrometry as previously reported (Tuck-

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[3] Citrosa Canada. 1991. Promotional literature. Citrosa Canada Inc., Upsala, Ontario.
[4] Horticultural DNA Products. 1990. Promotional literature. Horticultural DNA Products Inc., Miami, FL.
[5] Citrosa Canada. 1991. Advertising label included with citrosa plant. Citrosa Canada Inc., Upsala, Ontario.

Table 1. Mean percent composition (±SD) of essential oils[1] from greenhouse-grown (n = 6) and field-grown citrosa plants (n = 3).

Compound	Greenhouse-grown	Field-grown
Alpha-pinene	0.03 ± 0.01	0.08 ± 0.10
Myrcene	0.20 ± 0.07	0.30 ± 0.04
Alpha-phellandrene	1.12 ± 0.30	1.30 ± 0.16
Limonene	0.50 ± 0.53	0.28 ± 0.05
Beta-phellandrene	0.22 ± 0.05	0.35 ± 0.05
(*Z*)-beta-ocimene	0.15 ± 0.04	0.29 ± 0.08
(*E*)-beta-ocimene	0.06 ± 0.02	0.35 ± 0.09
p-cymene	0.07 ± 0.02	0.24 ± 0.03
Menthone	0.02 ± 0.02	0.27 ± 0.02
Citronellal	0.09 ± 0.05	0.07 ± 0.02
Alpha-copaene	0.30 ± 0.06	0
Isomenthone	7.63 ± 0.58	11.48 ± 0.93
Beta-bourbonene	0.05 ± 0.04	0.53 ± 0.08
Linalool	4.35 ± 0.56	11.56 ± 1.92
Alpha-guaiene	0.50 ± 0.12	0.83 ± 0.03
Beta-caryophyllene	0.26 ± 0.07	0.47 ± 0.02
Guaia-6,9-diene	6.15 ± 0.43	7.90 ± 0.14
Alpha-terpineol	0.20 ± 0.06	1.01 ± 0.13
Germacrene D	2.89 ± 0.39	2.73 ± 0.10
Citronellyl propionate	0.16 ± 0.12	0
Bete-selinene	0.11 ± 0.07	0.34 ± 0.03
Geranial	0.34 ± 0.21	0.36 ± 0.07
Bicyclogermacrene	0.03 ± 0.07	0.20 ± 0.05
Geranyl acetate	0.35 ± 0.11	0.24 ± 0.02
Citronellol	11.12 ± 1.32	8.81 ± 0.50
Nerol	0.24 ± 0.09	0.37 ± 0.02
Geranyl isobutyrate	0.34 ± 0.02	0.26 ± 0.04
Geranyl propionate	2.04 ± 0.44	1.26 ± 0.07
Geranyl butyrate	0.62 ± 0.09	0.86 ± 0.09
Furopelargone B	0.12 ± 0.24	0.07 ± 0.03
10-Epi-gamma-eudesmol	5.40 ± 0.57	ND[2]
Geranyl tiglate	3.83 ± 0.69	ND
10-Epi-gamma-eudesmol + geranyl tiglate	ND	5.39 ± 0.50
Phenylethyl tiglate	1.67 ± 0.16	0.94 ± 0.13
Decanoic acid	4.50 ± 1.2	0.89 ± 0.22

[1] Constituents listed in order of retention time during chemical analyses.
[2] ND = not determined.

er et al. 1991) and compared with commercial oils of rose geranium (variants of *P.* 'Rosé').

The repellency study was conducted in a flat, wooded area at the University of Guelph Arboretum in Guelph, Ontario, Canada (43°35′N, 80°20′W). Field tests were performed as a component of a larger study evaluating the effectiveness of various insect repellents (Surgeoner and Heal 1992). On either side of the experimental area were snowmelt pools (>1 ha) where large numbers of immature *Aedes* spp. develop. Although the understory within the study area varied slightly with location, the overhead canopy consisted of a uniform density of various deciduous species. Twelve locations were marked with numbered stakes placed at least 10 m apart on a grid measuring 30 × 30 m.

Over a 5-d period from June 10 to 14, 1991, 12 volunteer test subjects (7 males and 5 females) of varying weight (48–100 kg) and height (157–193 cm) between the ages of 21 and 33 were paired randomly and individually assigned to staked positions within the study site. Nontreated and 75% deet (*N,N*-diethyl-3-methylbenzamide) standard treatments were rotated throughout the sites on a daily basis such that each treatment was tested by 2 different individuals in 2 different sites each day. Citrosa plants were obtained from an Ontario plant nursery where plants were grown in a greenhouse. Although evaluated rel-

ative to the other treatments, citrosa subjects were not rotated and the same 2 individuals were used throughout the entire 5-d period. Plants were left *in situ* throughout the experiment to simulate a "backyard patio" situation.

Subjects were dressed in identical dark green coveralls and mosquito headnets. Sleeves were rolled up to the elbows such that hands and forearms were the only parts of the body exposed. Those testing the 75% deet (Nero Insect Repellent P.C.P. # 7137-04, Recochem Inc., Montreal, Quebec, H4T 1P4) applied 2 ml to their hands and forearms, whereas nontreated and citrosa individuals applied 2 ml of deionized water in the same manner. Citrosa "treatments" involved test subjects standing directly beside one potted 45-cm-high citrosa plant. Throughout the 12-h test period, individuals were asked not to wash their hands or engage in any strenuous physical activity. During each 5-min count, subjects aspirated all mosquitoes biting exposed hands and forearms. Mosquitoes were aspirated into 150-ml clear plastic vials with an X cut into the plastic lid that prevented escape. After 5 min the subjects sealed the plastic lid with tape, then recorded the number of mosquitoes captured. Each day, 30 mosquitoes were chosen randomly and identified to species using Wood et al. (1979).

This procedure was conducted for 4 5-min intervals for a total of 20 min of collecting at each of 4 sampling periods. After the first 2 intervals, individuals briefly walked around their plot to disturb any resting mosquitoes on surrounding vegetation. Biting counts were performed at 0730, 1130, 1530, and 1930 h Eastern Daylight Savings time each day for a total evaluation time of 12 h.

Percentage of protection was calculated as [(no. mosquitoes biting nontreated subjects − no. mosquitoes biting treated subjects)/(no. mosquitoes biting nontreated subjects)] × 100. Sampling periods in which 15 or fewer mosquitoes were collected by 2 nontreated controls were disregarded due to the severe influence of small numbers on percentage of protection. A test of the null hypothesis that the treatments did not significantly differ in repelling mosquito numbers was assessed by an analysis of variance using a 3-factor Model I 3 × 5 × 4 completely random design (Steel and Torrie 1980). Significant interactions between treatments, days, and sampling periods were also evaluated (Hubert and Hykle 1984). Rejection of the null hypothesis was followed by further analysis at each sampling period to determine consistency in treatment differences throughout the day. Significant differences in treatments were analyzed using a 2 tailed paired *t*-test. If data did not demonstrate a normal distribution and were positively skewed, values were log transformed and an initial F-test was performed. Significant differences in treatments were then determined by the least significant difference (LSD) method of analysis and completed using Statistical Analysis Systems version 6.04 (SAS Institute Inc., Cary, NC). All differences were considered significant at $P \leq 0.05$.

RESULTS

Pooling the essential oils of greenhouse-grown and field-grown plants, the essential oil of fresh "*Pelargonium citrosum*" had 35.4 ± 6.2% geraniol, 10.4 ± 1.6% citronellol, 8.9 ± 2.0% isomenthone, and 6.8 ± 3.8% linalool (Table 1). The total essential oil yield from the plants was 0.2 ± 0.1%. Components of the essential oil of citrosa were within the 95% range of those from commercial rose geranium oils (Table 2). In addition, levels of the leading components, geraniol and citronellol, were not significantly different from commercial rose geranium oils.

Relative to controls, 75% deet provided >90% protection after 8 h and >60% protection after 12 h, based on number of mosquitoes biting subjects (Table 3). At 0730 h, the number of mosquitoes caught did not differ between control and citrosa subjects (t = 1.32, 3 df). Subjects treated with 75% deet collected fewer mosquitoes than did controls or persons who stood next to the citrosa plant (t = 4.21 and t = 5.81, respectively, 3 df). At 1130, 1530, and 1930 h, data were positively skewed and initial F-tests indicated significant differences between deet, control, and citrosa treatments (F = 10.42, 5 and 6 df; F = 8.47, 4 and 4 df; F = 7.46, 6 and 8 df, respectively). For all sampling periods, the LSD method revealed significant differences between the deet and control and between the deet and citrosa treatments. No significant difference occurred between controls and citrosa treatment.

Analysis of variance also indicated significant interactions between treatment and day (F = 2.98, 8 and 60 df), day and sampling period (F = 3.95, 12 and 60 df), and treatment and sampling period (F = 3.93, 6 and 60 df). Significantly more mosquitoes were collected at 0730 and 1930 h, compared with 1130 and 1530 h. Although repellency provided by deet had decreased by 1930 h (Table 3), significantly fewer mosquitoes were collected by subjects treated with deet than by control and citrosa subjects.

Nine mosquito species were identified from 150 individuals collected throughout the 5-day study period. Species and percentage composition were *Aedes stimulans* (Walker) (30.0%), *Ae. canadensis* (Theobald) (25.3%), *Ae. euedes* Howard, Dyar, and Knab (18.6%), *Ae. fitchii* (Felt and Young) (14.0%), *Ae. cinereus* (Meigen) (3.3%),

Table 2. Mean percent composition (±SD) of essential oils[1] from greenhouse-grown 'Rosé' geranium (n = 3) and commercial rose geranium oil (n = 4).

Compound	Greenhouse-grown	Commercial oil
Alpha-pinene	0.12 ± 0.02	0.38 ± 0.44
Beta-pinene	0	0.18 ± 0.36
Myrcene	0.09 ± 0.01	0.08 ± 0.09
Alpha-phellandrene	0.08 ± 0.02	0.02 ± 0.03
Limonene	0.06 ± 0.01	0.38 ± 0.18
1,8-Cineole	0	0.10 ± 0.20
Beta-phellandrene	0.07 ± 0.02	0.03 ± 0.05
(*Z*)-beta-ocimene	0.08 ± 0.02	0.18 ± 0.23
(*E*)-beta-ocimene	0.01 ± 0.01	0.37 ± 0.33
p-cymene	0	0.10 ± 0.09
Terpinolene	0	0.09 ± 0.12
6-Methyl-5-hepten-2-one	0	0.08 ± 0.11
Cis-rose oxide	0.45 ± 0.09	0.36 ± 1.16
Trans-rose oxide	0.17 ± 0.03	0.63 ± 0.68
Santolina alcohol	0	0.62 ± 1.24
Cis-linalool oxide (furanoid)	0	0.39 ± 0.48
6-Methyl-5-hepten-2-ol	0	0.14 ± 0.28
Alpha-cubebene	0	0.02 ± 0.05
Trans-linalool oxide (furanoid)	0	0.13 ± 0.15
Menthone	0	1.76 ± 1.88
Citronellal	0.74 ± 0.10	0
Alpha-copaene	0.30 ± 0.02	0.19 ± 0.22
Isomenthone	4.47 ± 0.17	3.85 ± 2.79
Beta-bourbonene	0	0.68 ± 0.48
Linalool	1.39 ± 0.19	8.12 ± 4.16
Isopulegol	0	0.05 ± 0.06
Neomenthol	0	0.74 ± 1.48
Alpha-guaiene	0.88 ± 0.04	0.20 ± 0.40
Beta-caryophyllene	0.87 ± 0.06	1.48 ± 1.31
Guaia-6,9-diene	8.80 ± 0.27	2.59 ± 4.22
Citronellyl formate	19.36 ± 0.96	5.21 ± 4.52
Menthol	0	0.42 ± 0.70
Dihydrocitronellol	0	0.23 ± 0.47
Aromadendrene	0.29 ± 0.02	0.22 ± 0.44
Citronellyl acetate	0.46 ± 0.04	0.34 ± 0.25
Alpha-humulene	0.44 ± 0.23	0.09 ± 0.11
Neral	0.37 ± 0.01	0.08 ± 0.15
Alpha-terpineol	0	1.33 ± 1.01
Geranyl formate	0	1.32 ± 2.11
Viridiflorene	1.42 ± 0.10	0.09 ± 0.19
Germacrene D	3.69 ± 0.05	1.49 ± 2.34
Neryl acetate	0	0.32 ± 0.64
Citronellyl propionate	0.36 ± 0.08	0.16 ± 0.19
Alpha-muurolene	0	0.06 ± 0.13
Geranial	0.70 ± 0.03	0.42 ± 0.33
Geranyl acetate	0	4.32 ± 8.05
Citronellol	34.91 ± 0.91	20.82 ± 15.33
Gamma-cadinene	0	0.20 ± 0.40
Nerol	0	2.06 ± 2.59
Citronellyl butyrate	0.52 ± 0.04	0.39 ± 0.78
Geranyl isobutyrate	0	0.12 ± 0.23
Geranyl propionate	0.42 ± 0.06	0.63 ± 0.67
Geraniol	6.05 ± 0.39	22.57 ± 2.20
Geranyl butyrate	0	1.45 ± 0.90

Table 2. Continued.

Compound	Greenhouse-grown	Commercial oil
Beta-phenylethanol	0	0.07 ± 0.14
Alpha-calacorene	0	0.07 ± 0.14
Caryophyllene oxide	0	0.16 ± 0.33
Citronellyl tiglate	0	0.10 ± 0.20
Furopelargone B	0	0.25 ± 0.50
Geranyl n-hexanoate	0	0.08 ± 0.16
10-Epi-gamma-eudesmol	0	1.47 ± 1.71
Geranyl tiglate	1.52 ± 0.23	0.82 ± 1.64
Spathulenol	0	0.03 ± 0.06
Beta-phenylethyl tiglate	0	0.82 ± 0.69
Geranic acid	0	0.06 ± 0.12

[1] Constituents listed in order of retention time during chemical analyses.

Ae. excrucians (Walker) (3.3%), *Ae. punctor* (Kirby) (3.3%), *Ae. vexans* (Meigen) (1.3%), and *Ae. implicatus* Vockeroth (0.1%).

DISCUSSION

The cultivar 'Rosé' (perhaps more properly designated as a grex, or collection of related genomes) is part of the hybrid complex *Pelargonium* × *asperum* Ehrh. *ex* Willd., derived from *Pelargonium capitatum* (L.) L'Hér. *ex* Ait. × *Pelargonium radens* H. E. Moore (Van der Walt 1985, Van der Walt and Demarne 1988, Demarne and Van der Walt 1989). Many cultivars of this complex (e.g., 'Camphor Rose', 'Lady Plymouth', 'Peppermint Rose') have been incorrectly identified in previous literature as derived from *P. graveolens* L'Hér. *ex* Ait. Plants designated as "*P. citrosum*", or the citrosa plant, do not have any morphological characteristics, vegetative or floral, to differentiate them from the *P.* × *asperum* hybrid complex. "*Pelargonium citrosum*" is not a valid taxonomic designation.

Commercial citronella oils are of 2 types (Barber and Hall 1950). The oil of Mahapengiri or Java citronella from *Cymbopogon winterianus* Jowitt has 1.2 ± 0.6% linalool, 16.2 ± 2.8% citronellol + geranyl acetate, 22.2 ± 10.8% geraniol, and 20.3 ± 16.8% citronellal (Wijesekera 1973, Iruthayathas et al. 1977). The oil of Lenabatu or Ceylon citronella from *Cymbopogon nardus* (Linn.) Rendle has 1.1 ± 0.3% linalool, 12.5 ± 8.9% citronellol + geranyl acetate, 19.5 ± 1.3% geraniol, and 14.1 ± 15.5% citronellal (Wijesekera 1973, Manzoor-I-Khuda et al. 1982). Although both citronella oils are characterized by a high citronellal content, "*P. citrosum*" contained only trace amounts of this compound. Linalool is a major component of citrosa plants tested but is only a minor constituent of *C. winterianus* and *C. nardus* oils. The essential oil profile of citrosa more closely resembles that of other geranium oils and those analyzed here (Lawrence et al. 1975, Lis-Balchin 1991).

"*Pelargonium citrosum*" bears no unique morphological traits or essential oils to distinguish it from the *P.* × *asperum* complex. No characteristic morphology or essential oil of a *Cymbopogon* species yielding commercial citronella oil can be detected in citrosa. The citrosa plant is claimed in advertisement literature to repel mosquitoes by the release of the "fragrant oil of citronella"[6]. We observed that the undis-

[6] Austerica/Griffith. 1990. Promotional leaflet. Austerica/Griffith, Plymouth, FL.

Table 3. Mean number of mosquitoes (±SD) biting subjects (2 subjects per treatment) at 4 sampling periods, post-treatment. Values within columns sharing the same letter are not significantly different ($P \geq 0.05$).

Treatment	Time (h)			
	0730[1]	1130[1]	1530[1]	1930[2]
Nontreated	64.8 ± 30.8a	28.3 ± 20.6a	33.7 ± 19.7a	53.4 ± 12.7a
Citrosa plant	74.0 ± 25.5a	32.0 ± 24.8a	30.3 ± 32.3a	83.6 ± 46.7a
75% deet	0.0 ± 0.0b	0.8 ± 1.5b	2.7 ± 3.8b	18.8 ± 14.2b

[1] Based on 4 days evaluation.
[2] Based on 5 days evaluation.

turbed citrosa plant in our study did not add detectable fragrance to a room unless the plant was shaken or brushed with a hand. When placed outdoors, wind produced the same effect. Under field conditions, however, the citrosa plant did not protect human subjects from biting spring *Aedes* species. During field evaluations, mosquitoes were regularly observed landing and resting on the citrosa plant, indicating a lack of repellency. These findings support those of Cilek and Schreiber (1994) and Cummings and Craig (1995). In 1990, citrosa plants were introduced into Ontario, Canada, and retailed from $7.99 to $18.95, depending on size. The citrosa plant may be aesthetically pleasing and produce a pleasant scent when handled, however, citrosa plants should be advertised as such and not marketed as a mosquito repellent.

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