Efficacy of Several Commercially Formulated Essential Oils Against Caged Female *Aedes albopictus* and *Culex quinquefasciatus* when Operationally Applied via an Automatic-Timed Insecticide Application System

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EFFICACY OF SEVERAL COMMERCIALLY FORMULATED ESSENTIAL OILS AGAINST CAGED FEMALE *AEDES ALBOPICTUS* AND *CULEX QUINQUEFASCIATUS* WHEN OPERATIONALLY APPLIED VIA AN AUTOMATIC-TIMED INSECTICIDE APPLICATION SYSTEM

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ABSTRACT. The effectiveness of several commercially available products containing plant essential oils against caged female *Aedes albopictus* and *Culex quinquefasciatus* was studied. Products operationally applied via an automatic-timed insecticide application system (MistAway®) at maximum label rates were EcoExempt MC® (9.0 ml/liter, rosemary oil [18% AI], cinnamon oil [2% AI], lemongrass oil [2% AI], plus 78% "other ingredients" in wintergreen oil). Misting System Concentrate® (4 ml/liter, oil of *Juniperus virginiana* [85% AI]), Mosquito Barrier® (31.1 ml/liter, garlic oil [99.3% AI], and citric acid [0.5% AI]), and No-See-Um Organic Repellent® (99.5 ml/liter, lemongrass [4% AI], citronella [3% AI], castor oil [3% AI], sodium laurate [3%], and garlic oil [1% AI] in an 86% [AI] mixture of wintergreen oil, lecithin, and water). All products were compared with a commercial formulation of synergized pyrethrins, Riptide® (9.0 ml/liter, pyrethrins [5% AI], piperonyl butoxide [25% AI]), as a standard. Mortality was the greatest for Riptide to both mosquito species with effectiveness influenced by distance from the nozzle (ranging from >80% at 3 m to >10% at 20 m). The essential oil products resulted in <10% mortality for each species regardless of application exposure distance with the exception of EcoExempt, which gave ≈13% mortality of caged female *Ae. albopictus* 6 m from the nozzles.

KEY WORDS Essential oils, automatic mist systems, plant oils, 25(b) compounds

INTRODUCTION

A series of complex environmental, social, and economic policies have led to restrictions and general public aversion to the use of synthetic pesticides currently regulated by US federal and state authorities. Continued pressure is being exerted by antipesticide advocate groups to replace traditional insecticidal compounds with active ingredients containing "environmentally friendly" substances (e.g., essential oils) of often unknown efficacy. The US Environmental Protection Agency maintains a list of substances on their website that are considered exempt under 25(b) of the Federal Insecticide, Fungicide, and Rodenticide Act. This list consists of what are considered "minimum risk pesticides which is a special class of pesticides that are not subject to federal registration requirements because their ingredients, both active and inert, are demonstrably safe for the intended use" (US EPA 2009). Many of the active ingredients on this list are botanical essential oils. Although these products are considered to have minimal vertebrate toxicity, and therefore are generally regarded as safe by the US Food and Drug Administration (Kostyukovsky et al. 2002), their relative toxicity (i.e., efficacy) against mosquitoes is largely unknown when applied in an operational setting. Over the years, a considerable amount of work has been generated by several researchers in determining the effectiveness of several essential oils against a variety of public health and urban arthropod pests (Rani and Osmani 1984, Ngoh et al. 1998, Barnard 1999, Tarelli et al. 2009, Phillips et al. 2010). However, in regard to mosquito control, most essential oils of plants have primarily been evaluated as potential repellents or larvicides (see reviews by Jacobson 1958, Sukumar et al. 1991, and Barnard 1999) with sparse data on their effectiveness as adulticides (Yang et al. 2005, Chalyasit et al. 2006, Kang et al. 2009). Moreover, the operational efficacy of essential oils, especially commercial formulations for mosquito adulticides, is lacking. We have received increasing queries from the general public, and Florida mosquito control districts, about the effectiveness of essential oils in automatically timed insecticide application systems as substitutes for traditional insecticides currently labeled for their use. Automatically timed insecticide application systems, often referred to simply as "mist systems," apply insecticides at predetermined intervals (usually at dusk and dawn). These systems have gained considerable interest from homeowners for the purpose of controlling adult mosquitoes in residential backyards (Cilek et al. 2009). In this paper we report the efficacy of several commercially available products containing plant essential oils when applied under operational field conditions via a mist system against caged female *Aedes albopictus* (Skuse) and *Culex quinquefasciatus* Say.

MATERIALS AND METHODS

Materials tested

The following commercial products were applied as aqueous solutions at maximum label rates: EcoExempt MC® (rosemary oil [18% AI], cinnamon oil [2% AI], lemongrass oil [2% AI], plus 78% [AI] of "other ingredients" in wintergreen oil; applied at 34.4 ml/gallon [9.1 ml/liter]) (EcoSMART Technologies, Inc., Franklin, TN). EcoExempt Emulsifier® must be added to this product (according to the manufacturer's specifications) at a 1:1 ratio in order to fully disperse the latter product's essential oils into solution with water. We also evaluated Misting System Concentrate® (MSC) (oil of *Juniperus virginiana* L. [85% AI]; applied at 15 ml/gallon [4 ml/liter]) (CedarCide Industries, Inc., Spring, TX). EcoExempt MC and MSC were the only products we found that contained essential oils specifically labeled for use in automatically timed insecticide application systems. We also evaluated Mosquito Barrier® (garlic oil [99.3% AI] and citric acid [0.5% AI]; applied at 118 ml/gallon [31.1 ml/liter]) (Garlic Research Labs, Inc., Glendale, CA) and No-See-Um Organic Repellent® (lemongrass [4% AI], citronella [3% AI], castor oil [3% AI], sodium laurate [3% AI], and garlic oil [1% AI] in an 86% [AI] mixture of wintergreen oil, lecithin, and water; applied at 378 ml/gallon [99.5 ml/liter]) (Planet Natural, Bozeman, MT). Riptide® (pyrethrins [5% AI] and piperonyl butoxide [25% AI]; applied at 34 ml/gallon [9.0 ml/liter]) was used as a standard product that is labeled for use in automatic-timed insecticide application systems.

Study site

Adult mosquitoes were exposed to applications of the above products in wire cage bioassays in a simulated typical residential backyard previously described by Cilek et al. (2008). Briefly, the backyard was framed by a 1.2-m (height) polyvinyl chloride (PVC) pipe 15.2-m deep by 22.9-m wide perimeter "fence" arranged in the shape of an open rectangular "U". Two Mist-Away® automatic misting systems (MistAway Systems, Inc., Houston, TX) were used in this study. One system was a dedicated unit that only applied Riptide as a 0.05% AI finished aqueous solution from a 250-liter drum reservoir. The other system applied the essential oil products from a plastic 5-gallon (18.9 liter) bucket placed inside the 250-liter drum reservoir. To avoid cross contamination between treatments, separate buckets were used to dispense each product. Each spray system also consisted of a separate, continuous loop of 0.5-cm-diameter plastic tubing connected to 18 nondrip 0.30-mm SlimLine® nozzles (Natural Fog, Taiwan). Each nozzle was spaced 3.1 m (10 ft) apart and positioned on the inner top edge of the fence. Nozzles were oriented upward at ≈45°. According to the manufacturer, each nozzle delivered 41 ml/min at 180 psi. Each spray system automatically agitated its contents for at least 15 sec before application. A single line of 13.7-liter potted wax myrtle plants (*Myrica cerifera* L.) was placed along the inside perimeter of the PVC fence (total 85 plants) to simulate the vegetative border of a suburban backyard. Tops of the plants were ≈15.2 cm below the spray nozzles.

The morning before a treatment, the system was turned on and tested to ensure that fresh insecticide was in the line and that all nozzles were properly working. All treatments were randomly assigned as to day of application, and only 1 product was tested per day. Temperature, relative humidity, wind direction, and speed were all recorded during each test.

Wire cage mosquito bioassays

Mosquito cage bioassays used the methods previously reported by Cilek et al. (2008) where at least 15 laboratory-reared 5–7-day-old female *Ae. albopictus* and *Cx. quinquefasciatus* were mouth aspirated into 14 × 14 mesh vertical cylindrical copper wire cages (12-cm diameter × 13-cm length) with solid bottoms and a 1.8-cm-diameter hole to load mosquitoes. These mosquito species were used because of their importance as disease vectors (Mullen and Durden 2002). Cages were placed ≈1.5 m from ground surface on wooden stakes arranged in a grid starting 3.1 m away from the nozzles (42 cages total). Tests were repeated on 4 different calendar dates with each species in a separate cage but tested side by side. Misting system application consisted of a standard residential application time of 45 sec. Ten minutes after application (to allow enough time for the spray cloud to pass through the backyard) cages were removed from the treatment area. At that time mosquitoes were quickly removed from the cages, lightly knocked down with carbon dioxide, then transferred to clean 0.6-liter paper containers covered with fine screen cloth. A cotton ball soaked in a 10% aqueous solution of table sugar was placed on the top of each container. Mortality was assessed at 24 h. Mosquitoes were considered dead if they could not remain upright. Five untreated cages of each species were used as controls for each test. Control cages were positioned outdoors at the same time as testing but not in the test area and were similarly processed as the treatment cages.

To prevent nozzle cross contamination between essential oil treatments, the misting system was flushed at least 3 times with a 1% water solution of EcoExempt emulsifier followed by triple flushing with plain water the day before bioassays were to be conducted. After flushing, a wire bioassay cage of each mosquito species was placed within 1 foot of 5 spray nozzles (total 5 cages/species) and directly exposed to plain water spray for 45 sec. Mortality was assessed as above and averaged 0.9 ± 0.2% for *Ae. albopictus* and 0.4 ± 0.1% for *Cx. quinquefasciatus*.

Four replications of each product were conducted from August 25 through November 6, 2009, between 1700 and 1800 h. Wind speed averaged 3.3 ± 0.2 miles/h [5.3 ± 0.3 km/h] during testing, while temperature and relative humidity averaged 26.6 ± 1.0°C and 62.3 ± 3.9%, respectively.

Data analyses

Mean percentage mortality was calculated after correction for natural mortality in the controls (which averaged <3.5% for both species) using the formula by Abbott (1925). Data were then transformed via arc sine and subjected to an analysis of variance (ANOVA; SAS Institute 2002). Differences for each species and product between nozzle distance was determined by using the Student–Newman–Keuls multiple range test ($P \leq 0.05$) (Sokal and Rohlf 1981). Means of untransformed data are presented in Table 1.

RESULTS

Mosquito mortality was the greatest for Riptide (Table 1). Efficacy of this product was influenced by distance from the nozzle, where mortality was significantly greater at 3–9 m from the application nozzle for *Ae. albopictus* females compared with 12–20 m. The highest mortality of female *Cx. quinquefasciatus* in cages treated with Riptide was 81.8%. This level was significantly greater than most of the other distances except for cages at 6 m where there was no difference in mortality. Similarly, the highest mortality (88%) of *Ae. albopictus* occurred at 3 m but was not significantly different at 6 and 9 m. This drop off in efficacy, as a result of increasing distance, is similar to what we reported for these 2 species after similar exposure to SummerFrost (3% AI pyrethrins, 6% AI piperonyl butoxide, and 10% AI MGK-264) (Cilek et al. 2008).

For each mosquito species, the essential oil products generally resulted in <10% mortality regardless of application exposure distance (Table 1), with the exception of EcoExempt, which gave ≈13% mortality of caged female *Ae. albopictus* 6 m from the nozzles. Application distance did not significantly affect the level of mortality of either species for any of these products.

DISCUSSION

In general, the commercial botanical oil formulations used in this study were considerably

Table 1. Mean percentage (±SE) mortality at 24 h of caged female *Aedes albopictus* and *Culex quinquefasciatus* at varying distances from treatment nozzles exposed to a 45 sec application of Riptide©, EcoExempt MC©, Mosquito Barrier©, No-See-Um Organic Repellent©, or Misting System Concentrate© from a MistAway automatic-timed misting system in a simulated backyard.

Distance (m)	Riptide[1,2] *Ae. albopictus*	Riptide[1,2] *Cx. quinquefasciatus*	EcoExempt MC[1,3] *Ae. albopictus*	EcoExempt MC[1,3] *Cx. quinquefasciatus*	Mosquito Barrier[1,4] *Ae. albopictus*	Mosquito Barrier[1,4] *Cx. quinquefasciatus*	No-See-Um Organic Repellent[1,5] *Ae. albopictus*	No-See-Um Organic Repellent[1,5] *Cx. quinquefasciatus*	Misting System Concentrate[1,6] *Ae. albopictus*	Misting System Concentrate[1,6] *Cx. quinquefasciatus*
3	88.0 ± 2.2a	81.8 ± 2.9a	9.6 ± 0.1a	2.4 ± 0.1a	6.5 ± 1.5a	1.5 ± 0.5a	3.7 ± 0.8a	1.6 ± 0.3a	6.9 ± 2.5a	1.1 ± 0.3a
6	82.2 ± 3.1a	71.0 ± 3.1ab	13.1 ± 4.4a	1.0 ± 0.1a	7.6 ± 2.3a	2.3 ± 0.7a	3.9 ± 1.1a	1.2 ± 0.4a	9.6 ± 1.9a	1.0 ± 0.5a
9	82.4 ± 3.4a	45.3 ± 9.6bc	3.3 ± 1.1a	8.4 ± 0.5a	5.0 ± 2.0a	1.7 ± 0.7a	2.2 ± 0.6a	0.9 ± 0.6a	4.0 ± 2.1a	1.6 ± 0.8a
12	55.8 ± 4.6b	41.2 ± 8.5bc	6.4 ± 1.5a	0.1 ± 0.1a	1.6 ± 0.9a	1.7 ± 0.9a	3.5 ± 2.0a	2.0 ± 0.9a	2.6 ± 1.1a	0.3 ± 0.3a
15	59.2 ± 7.3b	19.0 ± 15.3c	5.5 ± 2.6a	0.1 ± 0.1a	0.7 ± 0.7a	3.1 ± 1.7a	1.4 ± 0.1a	1.3 ± 1.0a	5.7 ± 1.8a	0.3 ± 1.1a
20	37.8 ± 7.1b	10.9 ± 6.9c	0a	0a	0a	0a	3.3 ± 1.3a	2.6 ± 0.2a	1.4 ± 0.1a	1.3 ± 0.5a

[1] Means in each column followed by a different letter are significantly different ($P \leq 0.05$), Student–Newman–Keuls multiple range test.
[2] Pyrethrins (5% AI) and piperonyl butoxide (25% AI).
[3] Rosemary oil (18% AI), cinnamon oil (2% AI), and lemongrass oil (2% AI).
[4] Garlic oil (99.3% AI) and citric acid (0.5% AI).
[5] Lemongrass (4% AI), citronella (3% AI), castor oil (3% AI), sodium laurate (3% AI), and garlic oil (1% AI).
[6] Oil of *Juniperus virginiana* (85% AI).

less effective against caged female *Ae. albopictus* and *Cx. quinquefasciatus* when compared with Riptide. It is important to point out that Mosquito Barrier (garlic oil and citric acid) and Organic Repellent (lemongrass, citronella oil, castor oil, and garlic oil) were applied through the MistAway system at maximum label rates intended for application to vegetation as a barrier for mosquito control. Therefore, these 2 products can be considered to have been applied at considerably greater concentrations than the Riptide application.

Although some of the commercial botanical products that we tested contained ingredients considered to exhibit contact repellency to adult mosquitoes (e.g., citronella, garlic, etc.) we believe this did not have a bearing on the acute exposure results from our cage study. Moreover, some repellents have been reported to have toxic properties. Xue et al. (2003) found that aerosol applications of Skin-So-Soft®, containing 0.1% citronella, in laboratory wind tunnel studies produced 90% mortality of adult *Ae. aegypti* (L.) with 100% mortality of *Ae. albopictus* and *Anopheles quadrimaculatus* Say adults at 24 h posttreatment. In that same study these authors also similarly exposed these same species to Natrapel®, containing 10% citronella, where complete kill occurred at 24 h.

Although plant essential oils remain a promising source of compounds for control of some arthropod pests, most are not operationally useful against adult mosquitoes primarily due to very high median lethal doses compared with conventional adulticides. Indeed, as stated earlier in this paper, Mosquito Barrier (garlic oil and citric acid) and Organic Repellent (lemongrass, citronella, castor oil, and garlic oil), which were applied at 3.5 and 11 times, respectively, greater concentrations than Riptide, resulted in <10% mortality to either mosquito species. In summary, we found that the essential oil products evaluated in this study did not provide the level of adult mosquito control comparable with the standard synergized pyrethrins formulation when applied by an automatic-timed insecticide application system.

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