SEMI-FIELD ULV EVALUATION OF AN ALL-PURPOSE BOTANICAL INSECTICIDE CONTAINING CEDARWOOD AND CINNAMON OILS AGAINST ADULT *AEDES AEGYPTI*

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Subject editor: Eva Buckner

ABSTRACT

Public health mosquito control operates with only two classes of mosquito adulticides: pyrethroids and organophosphates. Recent work improving the emulsification of essential oils has increased the potential for development of plant-derived active ingredients. There is a growing body of literature on essential oils for various roles in mosquito management. NatureCide Pest Management (NCPM), a product available in private and commercial home pest control, uses a mixture of 25.3% cedarwood oil and 12.7% cinnamon oil as a Federal Insecticide, Fungicide, Rodenticide Act (FIFRA) exempt insecticide for both indoor and outdoor use. Recent investigations by the Anastasia Mosquito Control District of St. Johns County have found other FIFRA exempt products to be effective as a residual spray on vegetation. In continuing the exploration of botanical insecticides, NCPM was used in ULV tests against *Aedes aegypti* (L.) within its 35-122 ml per L of water label rate. Applications at 35 ml/L resulted in 60-70% knockdown after 1 hr and mortality after 24 hr. Increasing the rate to 70 ml/L resulted in 100% knockdown and mortality across all replications. Crystalline precipitation of the microemulsion was observed in mix tanks after standing for at least 2 wk, but it was not apparent that the efficacy of the product was reduced as a consequence. Cedarwood oil and cinnamon oil are a beneficial combination for ULV adulticiding against mosquitoes and could have a beneficial role for integrated mosquito management.

Key Words: *Aedes aegypti*, mosquito, botanical, insecticide, essential oils

INTRODUCTION

Botanical ingredients are attractive alternatives in formulated repellents (Gross and Coats 2015), toxicants (Gross et al. 2017), and synergists (Tong and Bloomquist 2013; Gross et al. 2017). The sustained demand for plant-derived active ingredients in pesticides has prompted the screening of over 350 plant essential oils as larvicides against *Aedes aegypti* (L.) (Dias & Moraes 2013). Phytochemicals have become increasingly viable for product development since successful formulation in microemulsions (Gross et al. 2017), and microemulsion formulations were demonstrated in pilot work as effective against *Culex pipiens* (Montefuscoli et al. 2013). In consequence, essential oils also are being screened as adulticides against *Ae. aegypti* and *An. gambiae* (Norris et al. 2015). Despite this effort, few products exist for mosquito management that use plant-derived active-ingredients, particularly for ultra-low volume (ULV) cold aerosol space sprays.

Amidst the emphasis on green chemistry underlies the principle cause of the demand for this research: EPA allows minimum risk pesticides to be exempt from FIFRA (40 C.F.R. §152.25 2015). This exemption is ideal for green products because environmental impact is minimal, and the product may be used more frequently than a FIFRA labeled product. This fundamentally appeals to desires for reapplication treatments when managing a significant mosquito outbreak or when mitigating arbovirus transmission. Furthermore, mosquito control is currently limited to two chemical classes for adulticides, which are the FIFRA regulated pyrethroids and organophosphates. However, exempt pesticides would provide different active ingredients for minimizing both resistance and environmental impacts.

One example of an exempt product, NatureCide Pest Management (NCPM), uses 25.3% cedarwood oil and 12.7% cinnamon oil as active ingredients. Cedarwood oil has been explored as a repellent against mosquitoes, ticks, and ants (Khanna and Chakraborty 2018; Eller et al. 2014), but has consistently shown high proclivity for killing arthropods, especially public health pests (Khanna and Chakraborty 2018; Eller et al. 2014; Singh et al. 1984). Cinnamon oil is an octopaminergic insecticide (Kostyukovsky et al. 2002) that expressed the greatest toxicity of eight adulticidal essential oils screened against adult *Culex quinquefasciatus* (Say) and *Musca domestica* (L.) (Benelli et al. 2018). It is also a synergist that increases the bioefficacy of other essential oils when presented together (Reegan et al. 2014).

The cedar and cinnamon oil mixture of NCPM is labeled for use against a variety of indoor and outdoor pests, including ants, fleas, filth flies, and other arthropods. Both of the aforementioned NatureCide products are not labeled for use as a space spray, instead being prescribed at rates for outdoor residual sprays. There is limited exploratory work with this and similar commercial products. However, utilization as a cold aerosol for ULV would provide more options to mosquito control. Therefore, we tested NCPM, which was recommended by the manufacturer for mosquito management, at the low end of its label rate to help determine the ULV potential of this alternative tool.

MATERIALS AND METHODS

The mosquito strain selected for testing was the 1952 Orlando strain *Aedes aegypti* sourced from the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology and reared in the insectaries of the Anastasia Mosquito Control District of St. Johns County. Mosquitoes were maintained at 26 ± 1.0°C, 65-80% relative humidity, and a photoperiod of 14:10 hr (L:D). The adult mosquitoes were provided 10% sugar solution as needed. Once mosquitoes were 5-7 d old, non-blood-fed females were selected for testing. To conduct assays, twenty females were transferred into cylindrical screened cages (4 x 10 cm) with the use of a HEPA-filtered mouth aspirator. Caged mosquitoes were acclimated to outdoor conditions for a minimum of 20 min prior to the start of any applications.

Treatments were carried out using NatureCide Pest Management (25.3% Cedarwood oil, 12.7% cinnamon oil, Pacific Shore Holdings, Inc., Canoga Park, CA). The label prescribed recommendation was to mix the product at a range of 35-122 ml per liter of water. For these tests, dilutions were arbitrarily selected at 35 ml/L and 70 ml/L. The formulation was applied by a truck-mounted single nozzle ULV cold aerosol sprayer (Guardian 95 ES, ADAPCO, LLC, Sanford, FL). The machine was calibrated to dispense droplets with an average size of 18 microns, spanning VMD of 10-30 microns (10 µ ≤ Dv 0.5 ≤ 30 µ), at 296 ml/min (10 oz/min). For each treatment, a row of polyvinyl chloride pipe stands, 1.2 m in height, held the mosquito cages mounted at 0.8-1 m above ground level. Stands were placed in three equidistant rows approximately 30 m, 60 m, and 90 m downwind from the truck drive path. Tests were conducted in the morning (0700 h-1100 h), with wind direction, wind speed, temperature, and relative humidity recorded on site. Spray trucks were driven at an average of 16 kilometers per hour in a straight line perpendicular to the length of the hanging field cage line. The treatment started 30 m prior to the first pipe stand and the treatment was shut off at 30 m past the last stand to ensure coverage during variable wind conditions. After the treatment, 15 min was allowed for drift to ensure passage of the spray plume downrange past the test plot before cages were gathered and returned to the laboratory for processing. Both dilutions were evaluated across three replications each. Once returned to the laboratory, mosquitoes were provided with 10% sucrose solution (in water) overnight using saturated cotton balls. Knockdown was recorded at 1 h and mortality was recorded 24 h post-treatment. Sets of 3 control cages per replicate were handled in an identical manner except being placed 30m upwind of the truck during application.

Data was corrected for control mortality below 10% by using Abbott's formula (Abbot 1925). Variation between field tested dilutions were analyzed in JMP 13.1.0 (SAS Institute, Inc., Cary, NC) using analysis of variance (ANOVA) and Tukey HSD test.

RESULTS AND DISCUSSION

Weather conditions averaged 27.5°C air temperature, 77.2% RH, and persistent south-southwest wind direction at 3.7 km/hr. Day conditions were clear and sunny with no persistent cloud cover or precipitation. Field assay data are summarized with mean and standard error of the mean (SEM) knockdown and mortality rates provided for 35 ml/L and 70 ml/L of NCPM in Fig. 1. There were no significant differences between the position in the 3 × 3 test array nor at discrete distances (30m, 60m, 90m) within knockdown ($F_{2,26}$ = 1.278, p = 0.3072) or mortality ($F_{2,26}$ = 2.4967, P = 0.1159) for 35ml/L. Treatments made at 70ml/L resulted in 100% knockdown and mortality at all distances and all replications (p < no variance). The lowest rate, 35 ml/L, averaged 60-70% knockdown

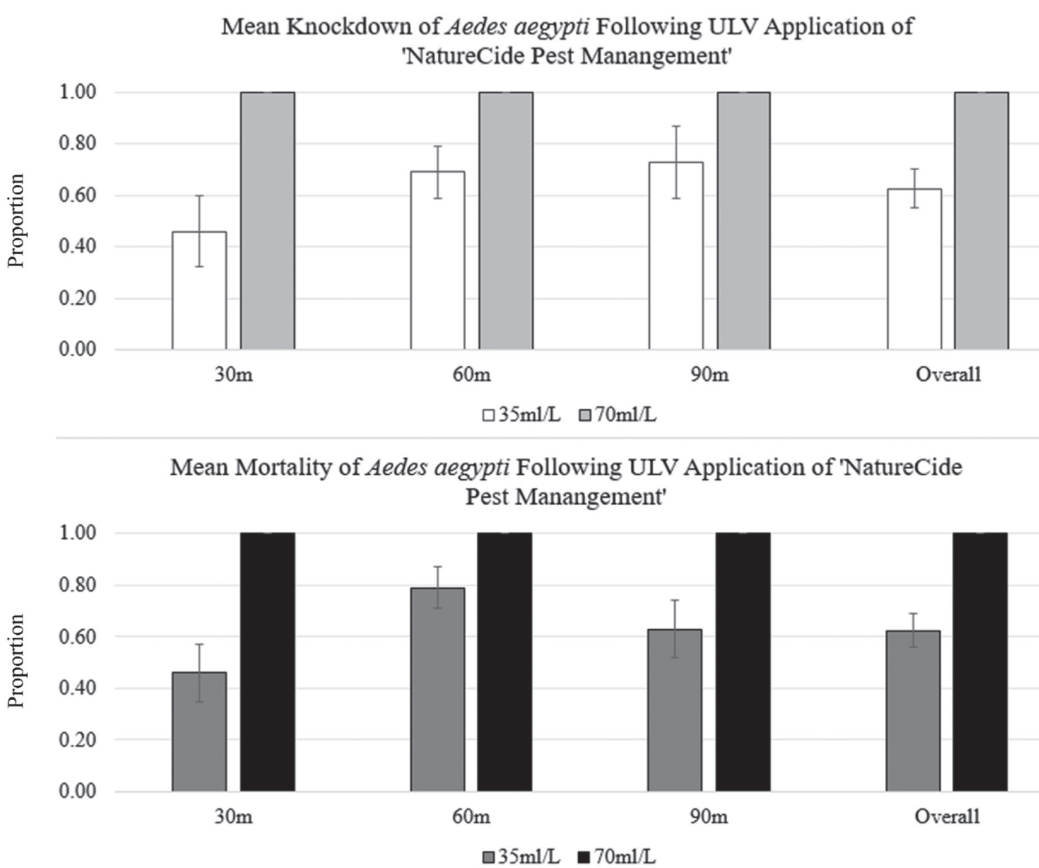

Figure 1. Significant mean (+ SEM) 1 hr knockdown and 24 hr mortality of *Aedes aegypti* (L.) were observed following ULV treatment with 35 ml/L and 70 ml/L of NatureCide Pest Management (25.3% cedarwood oil, 12.7% cinnamon oil) in a 3 × 3 grid with 30 m equidistant separations between mosquito cages (F = 5.34, df = 5, 54, p < 0.0005). There were no significant differences between the position in the 3 × 3 test array nor at discrete distances (30m, 60m, 90m) within knockdown ($F_{2,26}$ = 1.278, p = 0.3072) or mortality ($F_{2,26}$ = 2.4967, P = 0.1159) for 35ml/L. Treatments made at 70ml/L resulted in 100% knockdown and mortality at all distances and all replications (p < no variance). Treatments with 35 ml/L and 70 ml/L fell inside the low end of the label allowed rates of 35-122ml per liter of water.

and mortality among the exposed *Ae. aegypti* (Fig. 1). In contrast, all values for 70 ml/L were 100% for knockdown and mortality regardless of distance or position (Fig. 1). Knockdown and mortality were significantly greater at 70 ml/L than 35 ml/L, which was significantly greater than observed in the controls ($F = 5.34$, df = 5, 54, $p < 0.0005$). Control mortality was 0% in all trials.

Unexpectedly, crystalline precipitates were found on the surface of the liquid (Fig. 2) in the mix tank after the solution aged on the truck ULV assembly for 2 wk. Replicates using precipitated mixture were omitted from the data analysis, however there did not appear to be an obvious toxicity change when using precipitated mixtures. Freshly diluted product was used for each replicate and mix tanks were held for 6 wk after use. The crystalline precipitation occurred in all mixes regardless of which dilution. Agitation did not appear to resolve the precipitation of aged mixtures. Precipitation did not occur when mixtures were kept in cooler, laboratory conditions.

We intended to test farther into the label range for NCPM, however it was surprising to see it was not necessary to go higher than 70 ml/L, and perhaps not even necessary to go much higher than 35 ml/L. We did not test larvicide potential in this study, but it is also possible that exempt products made from botanical ingredients may be equally useful for larvicides as they are for adulticides (Norris et al. 2015). Furthermore, there may be additional benefits of NCPM in broader integrated management questions. Several examples of botanical oils for mosquito control are functional as repellents (Gross and Coats 2015) or synergists (Tong and Bloomquist 2013; Gross et al. 2017). Intensive screening of 361 essential oils from 269 plant spe-

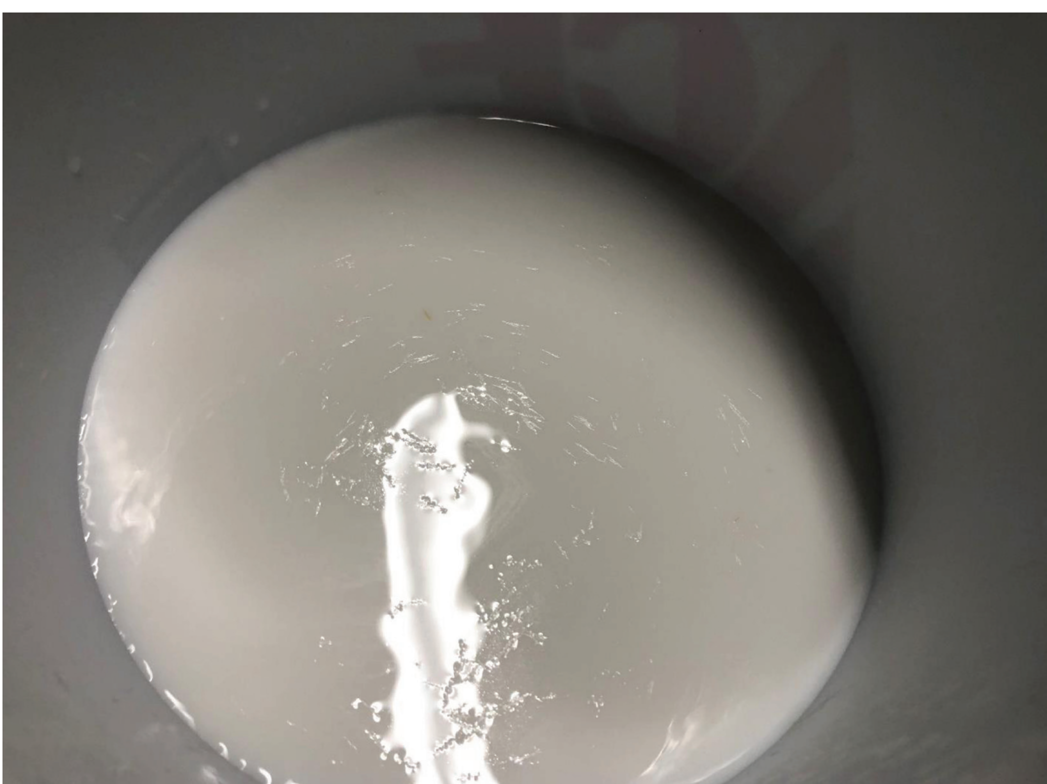

Figure 2. Crystalline precipitation in the mix tank for NatureCide Pest Management (25.3% cedarwood oil, 12.7% cinnamon oil) after 2 wk of storage after a replicate of truck mounted ultra-low volume cold aerosol treatment. Mixtures were left on the truck between the conclusion of treatment and the time of this image.

cies revealed dozens of potential larvicides against *Ae. aegypti* (Dias & Moraes 2013). Despite the aforementioned evidence, mosquito control has been slow to acquire botanical products for residual treatments or ultra-low volume (ULV) cold aerosol space sprays. By translating NatureCide Pest Management or similar products into public health operations, mosquito control can gain wider access to "green" alternative adulticides that do not have reapplication restrictions.

NatureCide Pest Management an EPA exempt product currently labeled for indoor and outdoor residual spot treatments against an assortment of urban and peridomestic insect pests. Meanwhile, the active ingredients, essential oils, appeal to eco-friendly proponents of botanical insecticides while still presenting a potentially effective mosquito adulticide. There may be broader utility in using these products if it also expands the circumstances or land area in which intervention can be made to reduce mosquitoes. The success of micro-emulsion formulations appears to be one reason that products may become more available from the discovered bioactive essential oils (Montefuscoli et al. 2013, Gross et al. 2017). Other FIFRA exempt products also have shown high comparative efficacy. Evaluation of an exempt sister product, NatureCide All-Purpose Commercial Concentrate containing clove and cottonseed oil, showed that when used as a vegetative barrier spray it outperformed Essentria IC[3] (rosemary oil, peppermint oil), Onslaught (fenvalerate), DeltaGard (deltamethrin), and performed equivalently with Cyzmic (lambda-cyhalothrin) (Smoleroff et al. 2019).

However, the stability of the micro-emulsions is not well understood in an operational context. The precipitation we observed in the mix tanks may imply that precautions need to be made with NCPM or similar essential oil emulsions if incorporating them into the machinery used in mosquito control operations. As an additional consideration, understanding non-target effects may in turn facilitate expansion of the label and trust in the blend of active ingredients in NCPM and similar products. Given the exemption status and consequent potential to reapply this insecticide frequently, it is critical to understand the non-target impacts of application on key pollinators or to water ecology. Regardless of the gaps in knowledge, we believe our positive results using NatureCide Pest Management as a ULV treatment highlights that some botanicals are ready to be incorporated into mosquito control programs.

ACKNOWLEDGEMENTS

We would like to thank Jason Conrad and Univar for their assistance with selecting and sourcing the product chosen for this study. This is a research report only. Specific mention of any commercial products does not imply endorsement by the Anastasia Mosquito Control District.

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