Mode of Action for Natural Products Isolated From Essential Oils of Two Trees Is Different From Available Mosquito Adulticides

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ABSTRACT Insecticidal properties of natural products may present alternatives to the use of synthetic molecule pesticides that are of diminishing effectiveness due to resistance. Three compounds, thymoquinone, nootkatone, and carvacrol, components of Alaska yellow cedar, *Chamaecyparis nootkatensis* (D. Don) Spach, and incense cedar, *Calocedrus decurrens* (Torr.), essential oils, have been shown to have biological activity against a variety of mosquito and tick species. Although these components act as both repellents and insecticides, how they function in either capacity is unknown. Their use as mosquito control insecticides would be greatly increased if their mode of action is not the same as that of currently used commercial products. This study compared the lethal dosages for nootkatone, carvacrol, and thymoquinone by using colony strains of *Anopheles gambiae* Giles with known mutations at three different target sites. The altered target sites evaluated were the sodium channel para-locus mutation (L1014 F KDR) that confers permethrin resistance, the ACE-1 gene that confers organophosphate and carbamate resistance, and a γ-aminobutyric acid receptor mutation of the Rdl locus conferring dieldrin resistance. Significant increases in lethal dose were not observed in any of the mosquito strains for any of the compounds tested compared with the doses required of chemicals with known modes of action at the mutated sites. Although the mode of action was not determined, this screening study indicates that none of these compounds interact at the target sites represented in the test mosquito strains. These compounds represent a different mode of action than existing chemicals currently used in mosquito control.

KEY WORDS mode of action, mosquitoes, nookatone, thymoquinone, carvacrol

Commercial insecticides used in mosquito control have traditionally come from those developed for agriculture. In the United States, insecticides used for adult mosquito control come from two classes of chemicals, pyrethroids and organophosphates. Outside of the United States, additional classes may sometimes be used such as organochlorines, carbamates, and cyclodienes. All of these chemical classes rely on three primary target sites within the insect vector: the gated sodium channel (pyrethroids and DDT), inhibition of γ-aminobutyric acid (GABA) receptors (cyclodienes), and the inhibition of acetylcholinesterase (organophosphates and carbamates) (Ware 1991).

Resistance in vector mosquitoes has been documented to occur throughout the world in a variety of important species (Hemingway and Ranson 2000, Xu et al. 2006). With only two primary modes of action available for vector control of adult mosquitoes in the United States (i.e., the gated sodium channel and inhibition of acetylcholinesterase), the development of resistance in an area can critically affect vector control. Finding suitable chemicals with alternative modes of action is of urgent concern among vector control personnel.

Essential oils of many plant species have been investigated as possible alternatives of traditional chemicals based on the assumption they are natural and thus safer (Coats 1994, Isman 2006). Essential oils of two North American tree species, Alaska yellow cedar, *Chamaecyparis nootkatensis* (D. Don) Spach, and incense cedar, *Calocedrus decurrens* (Torr.), have been investigated for their insecticidal and acaricidal properties (Panella et al. 2005, Dolan et al. 2007). Their results have shown essential oils of both species have insecticidal and repellent properties, albeit at lower levels than currently available compounds. Our study was conducted to evaluate whether three components of these tree essential oils, thymoquinone, nootkatone, and carvacrol, have similar modes of action to currently available mosquito adulticides. Possessing a different mode of action would make these compounds more valuable for further commercial development as they would be particularly beneficial in areas with documented insecticide resistance.

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Methods and Materials

Mosquitoes. Four strains of Anopheles gambiae Giles were procured from the malaria research and reference reagent resource center (MR4) program, part of the American Type Culture Collection (Manasses, VA). Three strains have insecticide resistance characterized by altered target sites and the fourth is insecticide susceptible. The P+DLRC+R+ strain has an alanine-to-glycine mutation in the Rdl locus coding for a γ-aminobutyric acid (GABA) receptor conferring resistance to dieldrin (Du et al. 2005). The RSP-ST strain contains a leucine-phenylalanine substitution at position 1014 (L1014 F KDR) of the voltage-gated sodium channel allele (Ranson et al. 2000) and other unidentified resistance mechanisms (Vulule et al. 1999) conferring pyrethroid resistance. The AKRON strain has resistance conferred to carbamates by an insensitive ACE-1 mutation and also possesses LL1014 F KDR. The G3 wild type is a susceptible strain in colony since 1975.

All mosquito colonies were maintained at 27–28°C, 85–90% RH, and a photoperiod of 12:12 (LD) h. All adults were allowed to feed on a 10% sucrose solution ad libitum. Adult females were provided defibrinated calf blood (Colorado Serum, Denver, CO) by using a Hemotek membrane feeding system (Discovery Scientific, West Chester, PA) were tested on the RSP-ST, P+DLRC+R+, and AKRON strains, respectively, and compared with results from testing each chemical on G3 (susceptible colony). Control mortality was recorded after 24 h. The compounds tested were nootkatone (Frutarom, North Bergen, NJ), thymoquinone (TCI America, Portland, OR), and carvacrol (TCI America).

To confirm the level of resistance in the resistant colonies, permethrin, dieldrin, and propoxur (ChemService, West Chester, PA) were tested on the RSP-ST, P+DLRC+R+, and AKRON strains, respectively, and compared with results from testing each chemical on G3 (susceptible colony). Control mortality was corrected using Abbott’s formula as modified and provided by the WHO (1998). Lethal dosages were calculated and chi-square goodness-of-fit tests were performed using SAS software (PROC PROBIT, SAS Institute 2003).

Table 1 summarizes the results for all the tests. The LD₅₀ value for thymoquinone ranged from 0.933 µg/mg (95% CI, 0.875–0.995 µg/mg) in RSP-ST to 1.502 µg/mg (CI, 1.481–1.524 µg/mg) in AKRON. The range of LD₅₀ value for nootkatone was 1.460 µg/mg (CI, 1.190–1.726 µg/mg) in G3-2.502 (CI, 2.236–2.775 µg/mg) in AKRON. The carvacrol LD₅₀ value ranged from 0.683 µg/mg (CI, 0.675–1.905 µg/mg) in RSP-ST to 2.649 µg/mg (CI, 2.378–2.905 µg/mg) in G3. The LD₅₀ value for permethrin was 0.000214 µg/mg in G3 and 0.00385 µg/mg in RSP-ST. Propoxur had an LD₅₀ value of 0.000719 µg/mg in G3.
µg/mg in G3 and 3.628 µg/mg in AKRON, whereas for dieldrin the LD50 value was 0.00455 µg/mg in G3 and 6.628 µg/mg in P+DLRC+R+.

Resistance ratios (RRs) were calculated for each compound tested. A ratio of <1 indicates the “resistant” strain is more susceptible than the “susceptible” strain; however, in this study all RRs were close to 1 for the tree oil components. The thymoquinone RR was 0.65, 0.82, and 1.06 in RSP-ST, D+DLRC+R+, and AKRON, respectively. Nootkatone RR was 1.12, 1.47, and 1.71, and carvacrol RR was 0.65, 0.78, and 0.79 for the same mosquito strains in the same order.

This contrasts dramatically with the RRs calculated for the resistant strains and insecticides known to affect the target sites containing the mutations conferring resistance. Propoxur, a carbamate, inhibits acetylcholinesterase; dieldrin, a cyclodiene, acts by inhibiting GABA; and permethrin, a pyrethroid, works on the gated sodium channel (Ware 1991). The RR forpermethrin in the RSP-ST strain with resistance to pyrethroids was 16.42. The RR for propoxur was 5.046 in the AKRON strain with organophosphate and carbamate resistance. The RR for dieldrin in the P+DLRC+R+ strain with cyclodiene resistance was 1,457.

It was expected that if any of the three tree compounds, thymoquinone, nootkatone, or carvacrol, were affecting one of the target sites represented in the three resistant strains then a similar greatly increased RR would be observed. Because this was not the case and all resistance ratios were close to 1, it can be inferred that their mode of action is different from pyrethroids, organophosphates, carbamates, and cyclodiene. This represents a significant finding as these compounds may prove invaluable in maintaining our ability to control populations of mosquitoes that are resistant to currently available insecticides. However, formulations, best application methods and environmental studies still need to be conducted before they are commercialized and registered.

Any commercial product developed for vector control using thymoquinone, nootkatone, or carvacrol would be extremely beneficial for resistance management. Although we now know these compounds are not affecting the same target sites as existing vector control insecticides, there are many other commercially available insecticides for plant pests that also have alternative modes of action. For example, abamectin is a chloride channel activator and imidacloprid and spinosad are nicotinic acetylcholine receptor agonists (Pridgeon et al. 2008). Spinosad has recently been registered in the United States as a mosquito larvicide under the trade name Natrural (Clarke, Roselle, IL). The toxicity of imidacloprid and abamectin has been evaluated as possible mosquito adulticides (Pridgeon et al. 2008). Agriculture uses of insecticides can impact the development of resistance in vector species, but in most cases the documented resistance is to insecticides that are already in the same class as those used for vector control (Mouchet 1988, N’Guassen 2003).

Development of these compounds into commercially available products would give vector control new products with alternative modes of action from those currently available. These products also would be novel in that they were developed for vector control and not adapted from existing agriculture uses. Furthermore, they have the potential of being more readily acceptable by the public because of their origins from natural sources.

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