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Impact of Pyrethroid Resistance on the Intrinsic Insecticidal Activities of Geraniol Against the Yellow Fever Mosquito, *Aedes aegypti*

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Simple Summary

The present study compared the toxic and repellent effects of geraniol on *Aedes aegypti* mosquitoes from representative pyrethroid-susceptible and pyrethroid-resistant strains. Although the pyrethroid resistant strain showed modest resistance to geraniol as a toxin and repellent, the degree of resistance was far lower than that for a pyrethroid or pyrethrum extract. Our results suggest that geraniol has potential as a chemical tool for controlling pyrethroid resistant populations of *Ae. aegypti*.

Abstract

The evolution of resistance in mosquitoes to conventional pesticides such as pyrethroids presents a challenge to vector control. Thus, alternative active ingredients for pesticides to manage pyrethroid resistant populations of mosquitoes are needed. The goal of this study was to evaluate the toxic and repellent efficacies of geraniol, a plant secondary metabolite, as a potential alternative for controlling pyrethroid-resistant *Aedes aegypti*. We found that addition of geraniol to rearing water of 1st instar larvae caused concentration-dependent mortality within 24 h in both strains. The resistance ratio of geraniol (2.8) was modest compared to that of cypermethrin (435.3). Topical application of geraniol to adult female mosquitoes caused dose-dependent mortality in both strains within 24 h. The resistance ratio of geraniol (1.1) was minimal compared to that for cypermethrin (457). In spatial repellency assays, geraniol repelled adult females from both strains in a dose-dependent manner. The repellency resistance ratio of geraniol (2.6) was modest compared to that for pyrethrum extract (>132). Our findings suggest that geraniol has potential use as a versatile chemical tool for controlling pyrethroid-resistant populations of *Ae. aegypti*.

Keywords: mosquito; geraniol; larvicide; adulticide; repellent

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1. Introduction

Mosquito-borne arboviruses such as dengue, Zika, and chikungunya represent significant global health threats, collectively infecting hundreds of millions of people and causing tens of thousands of deaths annually [1–3]. *Aedes aegypti*, the yellow fever mosquito, is one of the most important vectors for transmission of these viruses to humans. Control strategies for *Ae. aegypti* have relied on chemical approaches that use insecticides with limited modes of action (e.g., pyrethroids), which has led to insecticide resistance in mosquito populations and challenges to the control of mosquito-borne arboviral diseases [4–6]. Pyrethroid resistance in mosquitoes can arise through multiple mechanisms, including target-site insensitivity (e.g., knockdown resistance, *kdr*, mutations in voltage-gated sodium channels) and metabolism (e.g., overexpression of detoxification enzymes, such as cytochrome P450 monooxygenases) [7,8].

In addition, overuse of some insecticides (e.g., pyrethroids), has raised concerns about threats to non-target species and environmental health. Pyrethroids act on neural signaling proteins, the voltage-gated sodium channels, which are conserved across other arthropods and vertebrates,

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including humans [4,9–11]. As a result, there is a need to develop new environmentally-friendly chemical tools for vector control.

Plant essential oils and their constituents represent a promising source of bioactive compounds for developing new pesticides. Essential oils are rich in secondary metabolites, which often play crucial roles in the chemical defenses of plants [12–16]. Some secondary metabolites exhibit insecticidal activity, making them potential active ingredients for developing biopesticides. Furthermore, some secondary metabolites are considered safer for human health, less harmful to non-target organisms, and more environmentally sustainable than conventional synthetic insecticides [12,14].

Geraniol is a common, abundant secondary metabolite found in essential oils of several plants, including geranium, citronella, and rose [17–20].This compound is a monoterpene alcohol with a rose-like scent that is listed by the U.S. Environmental Protection Agency (EPA) as a minimum risk pesticide [21–24]. Essential oils rich in geraniol have larvicidal and ovicidal effects against several species of mosquitoes, including *Culex pipiens*, *Culex quinquefasciatus*, *Aedes albopictus*, and *Ae. aegypti* [25–28]. Consistent with these studies, geraniol alone has been demonstrated to show larvicidal activity against multiple mosquito species, including *Cx. pipiens*, *Cx. quinquefasciatus*, *Anopheles stephensi*, *Ae. albopictus*, and *Ae. aegypti* [21,25,26,29,30]. Moreover, geraniol and/or essential oils enriched with geraniol have been shown to elicit adulticidal activity [28,31]. However, a limitation of these prior studies is that they were conducted primarily on insecticide-susceptible mosquito strains. Thus, the toxic efficacy of geraniol against insecticide-resistant mosquitoes is underexplored.

Prior studies have also investigated geraniol's potential use as a mosquito repellent, which has yielded mixed results. For example, some studies have found that geraniol is a relatively strong repellent, outperforming other natural repellents such as citronella and linalool against susceptible strains of *Ae. aegypti* [14,24]. However, another study found geraniol to be an ineffective repellent against *Ae. aegypti* [32]. Intriguingly, in *An. gambiae*, geraniol showed similar repellent efficacy against both a pyrethroid susceptible and pyrethroid resistant strain, but weaker repellency against an organophosphate-resistant strain [4]. Thus, our understanding of the potential use of geraniol as a repellent is limited and requires further investigation, especially against insecticide resistant mosquito strains.

The goal of this study is to address the above research gaps by comparing the larvicidal, adulticidal, and repellent effects of geraniol between a pyrethroid-susceptible and a pyrethroid-resistant *Aedes aegypti* strain. Given that a previous computational study [33] predicted that geraniol interacts with voltage-gated sodium channels as well as certain detoxification enzymes involved in pyrethroid resistance (e.g., glutathione *S*-transferase, esterase) in *Ae. aegypti*, we hypothesize that pyrethroid-resistant *Ae. aegypti* will exhibit toxic resistance to geraniol. On the other hand, given that geraniol was equally effective as a repellent against pyrethroid-susceptible and pyrethroid-resistant strains of *Anopheles gambiae*, we hypothesize that pyrethroid-resistant *Ae. aegypti* will show limited repellency resistance to geraniol.

2. Materials and Methods

2.1. Aedes aegypti Colonies and Strains

Two different strains of *Aedes aegypti* mosquitoes were used in the present study: Liverpool (LVP-IB12, MRA-735, obtained by David W. Becnel) and Puerto Rico (PR-NR-48830, obtained by G.G. Clark & J.J. Becnel). The Puerto Rico strain is highly resistant to pyrethroids via both target site (*kdr*) and metabolic resistance [34–36]. From here, we refer to the Liverpool strain as pyrethroid-susceptible (PS) and the Puerto Rico strain as pyrethroid-resistant (PR). To rear mosquitoes, larvae from both strains were fed with 1 tablet per day of commercial fish food (Tropical Tablets, Tetramin, Blacksburg, VA, USA). Adult mosquitoes were maintained on 10% sucrose; for egg production, adult females were fed defibrinated rabbit blood (Hemostat Laboratories, Dixon, CA) via a membrane feeder (Hemotek Ltd., Blackburn, UK). All mosquito colonies were maintained in environmentally-

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controlled rearing chambers set at the following conditions: 28 °C, 80% relative humidity, and 12:12 light: dark cycle.

2.2. Chemicals

Geraniol (≥ 99%, (2E)-3,7-dimethylocta-2,6-dien-1-ol) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Cypermethrin (>96%) was obtained from Thermo Fisher Scientific (Acros Organics, Geel, Belgium). Transfluthrin (≥99%) was obtained from Thermo Fisher Scientific (Chicago, IL, USA). Pyrethrum extract (≥50% sum of pyrethrins) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Larvicidal Bioassay

Larvicidal activities of geraniol and cypermethrin were tested against 1st-instar larvae of PS and PR strains using an established bioassay [13,37]. Cypermethrin was used as a positive control/reference compound for confirming toxic resistance of larvae of the PR strain [37,38]. In brief, 5 larvae were added to wells of 24-well Falcon Multiwell plates (Becton Dickinson Labware, Franklin Lakes, NJ, USA) containing 985 μL of distilled water and 5 μl of a food suspension. The food suspension consisted of 13 mg of finely ground fish food flakes (Tetramin, Blacksburg, VA, USA) suspended in 1 mL of deionized water. After adding larvae, solvent control wells received 10 μL of 100% acetone, whereas treatment wells received 10 μL of geraniol or cypermethrin dissolved in 100% acetone at various concentrations (freshly prepared on the day of testing). The final concentrations of geraniol in the wells were 1.2, 3.7, 11, 33, or 100 ppm; the final concentrations of cypermethrin in the wells were 0.0004, 0.004, 0.04, 0.4, 4.0, or 40 ppm. The plates were held in the rearing chambers and larval mortality was assessed 24 h post-treatment. Larvae were classified as dead if they failed to respond after gently probing with a pipette tip. Each control and chemical concentration was tested in quadruplicate wells on 8 independent plates (32 wells total) for each strain.

2.4. Topical Adulticidal Bioassay

Topical adulticidal activities of geraniol and cypermethrin were tested against adult females of PS and PR strains using an established bioassay [39,40]. Cypermethrin was used as a positive control/reference compound for confirming toxic resistance of adult females of the PR strain [38]. Ten mosquitoes (3-10 days post-eclosion) were briefly immobilized on ice and 500 nL of 100% acetone (solvent control) or a treatment (geraniol or cypermethrin dissolved at various concentrations in 100% acetone) were applied to the thorax of each mosquito using a Hamilton repeating dispenser (Hamilton Company, Reno, Nevada). The doses of geraniol were 0.20, 0.55, 1.85, 5.50, and 16.5 μg/mosquito; the doses of cypermethrin were 0.00019, 0.0019, 0.019, 0.19, 1.9, and 19.2 ng/mosquito.

After treating the mosquitoes, they were transferred to small cages (32 oz. containers) and provided with access to 10% sucrose. The cages were held in the rearing chamber and toxicity was evaluated based on the percentage of mosquitoes that were dead 24 h post-treatment. Each dose of geraniol and cypermethrin was tested on 5 independent cages of 10 mosquitoes each.

2.5. Mosquito Airborne Repellency Test (MART Assay)

Spatial or airborne repellency of geraniol and pyrethrum extract were tested using a previously described method [41], with the minor modification that 12 mosquitoes were used per replicate instead of 16. Transfluthrin was initially used as a positive control/reference compound for confirming repellency resistance of adult females of the PR strain [42]. However, in preliminary experiments, we did not detect repellency of this compound against either PS or PR strain, which may have been attributed to its vapor toxicity, resulting in immobile or knocked down mosquitoes. Thus, we switched to pyrethrum extract as a positive control/reference compound for repellency [42]. Groups of twelve adult females were transferred into glass cylinders (length 12.5 cm, outer diameter 2.5 cm; TriKinetics Inc, Walthman, MA, USA) with mesh netting secured on both ends with rubber

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bands (the netting was temporarily removed from one end to introduce the mosquitoes). Before adding the mosquitoes, a line was drawn on each tube at the half-way point of its length. Mosquitoes were acclimated in the tubes for 30 min in a rearing chamber (28 °C, 80% relative humidity) before starting experiments.

While mosquitoes were acclimating to the tubes, fifty microliters of 100% acetone (solvent control) or a treatment (geraniol or pyrethrum extract dissolved in 100% acetone) were applied to round filter papers (diameter 2.5 cm; Cytiva, Fisher Scientific, Waltham, MA, USA). Geraniol was applied at 0.5, 1.0, 3.8, 12.0, and 38.0 μg/cm^2 for experiments on PS mosquitoes, and 3.8, 12.0, 38.0, 112.0, and 336.0 μg/cm^2 for experiments on PR mosquitoes. Pyrethrum extract was applied at 1.1, 3.3, 12.0, 33.0, and 127.3 μg/cm^2 for experiments on PS mosquitoes, and 101.0, 254.6, 336.0, 509.2, 1,018.3, and 2,036.7 μg/cm^2 for experiments on PR mosquitoes. The filter papers were placed in plastic caps from 50 mL polypropylene centrifuge tubes (VWR, Radnor, PA USA) under a fume hood for 10 min to allow acetone to evaporate.

To initiate an experiment, one end of a glass tube containing mosquitoes was fitted with a cap containing the treatment filter paper, whereas the other end was fitted with a cap containing the solvent control filter paper. In addition, to confirm that mosquitoes showed no preference towards either end of the tube, double-solvent controls were performed in which both ends were fitted with caps containing filter papers treated with 100% acetone. Fifteen minutes after fitting the caps, the number of mosquitoes on the treatment and control halves of the tubes were counted and the repellency of the treatment was calculated using Formula 1. Any mosquitoes that were knocked down (i.e., not upright, suggesting intoxication or poor health) were excluded. In the double-solvent controls and pyrethrum extract experiments, no knock down of mosquitoes was observed. In geraniol experiments, a few individuals of the PR strain were knocked down at the two highest doses. That is, in the 112.0 μg/cm^2 treatment, knocked-down mosquitoes were observed in 2 of 6 replicates (1 or 2 individuals). In the 336.0 μg/cm^2 treatment, a few knocked-down mosquitoes were observed in each replicate: 2 individuals in 5 of the 6 replicates and 1 knocked-down individual in 1 of the 6 replicates. The double-solvent controls for each experiment showed minimal repellency for both the PS and PR mosquitoes (Figure S1), indicating no preference of mosquitoes towards either end of the tubes in the absence of geraniol or pyrethrum extract.

Formula 1.

$$\text{Repellency (\%)} = \left(\frac{\#\ of\ mosquitoes\ on\ control\ side - \#\ of\ mosquitoes\ on\ treatment\ side}{total\ \#\ of\ mosquitoes}\right) \times 100$$

2.6. Statistical Analysis

All data were analyzed using Prism 10 (GraphPad Software). For toxicity assays, the median lethal concentrations (LC_{50}) for larvae or doses (LD_{50}) for adults were determined by plotting percent mortalities against log transformations of the treatment tested and fitting a non-linear regression (log(agonist) vs. normalized response function). For repellency assays, the median effective doses (ED_{50}) were calculated similarly. Statistical comparisons of the LC_{50}, LD_{50}, or ED_{50} values between the PS and PR strains were compared through extra sum-of-squares F-tests ($\alpha = 0.05$).

3. Results

3.1. Geraniol and Cypermethrin Toxicity Against PS and PR Larvae

Addition of geraniol to the rearing water of first-instar *Ae. aegypti* larvae resulted in concentration-dependent mortality within 24 h in both the PS and PR strains (Figure 1a). The LC_{50} of geraniol for the PS strain (13.6 ppm, 95% CI: 11.0–16.6 ppm) was lower ($p < 0.0001$) than that of the PR strain (38.6 ppm, 95% Cl: 33.7–44.1 ppm), indicating a larvicidal resistance ratio of 2.8. To provide context for the larvicidal resistance ratio of geraniol, similar experiments were performed using cypermethrin. Previously, we found the resistance ratio of larvae from this same PR strain ranged from 30 to 131 for cypermethrin [37,38]. As shown in Figure 1b, the 24 h LC_{50} of cypermethrin for the PS strain in the present study was 0.02 ppm (95% CI: 0.01–0.04 ppm), whereas that for the PR strain

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was dramatically higher ($p < 0.0001$) at 7.40 ppm (95% CI: 1.01–10.16 ppm), indicating a resistance ratio of 435.3.

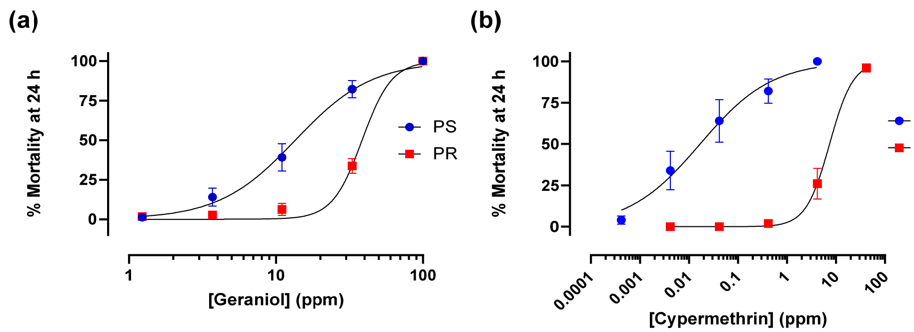

Figure 1. Concentration-mortality (24 h) curves for **(a)** geraniol and **(b)** cypermethrin against 1st instar larvae of pyrethroid-susceptible (PS, blue) and pyrethroid-resistant (PR, red) *Ae. aegypti*. For geraniol**,** values are means ± SEM based on 8 replicates per concentration for the PS strain and 5 replicates per concentration for the PR strain. For cypermethrin, values are means ± SEM based on 5 replicates per concentration for both the PS and PR strains. No mortality was observed in solvent controls.

3.2. Geraniol and Cypermethrin Toxicity Against PS and PR Adult Females

Topical application of geraniol to adult female *A. aegypti* resulted in dose-dependent mortality within 24 h in both the PS and PR strains (Figure 2a). The LD_{50} for the PS strain (6.5 µg/mosquito, 95% CI: 5.1–8.3 µg/mosquito) was similar ($p = 0.6$) to that for the PR strain (7.1 µg/mosquito, 95% CI: 5.7–8.8 µg/mosquito), indicating an adulticidal resistance ratio of 1.1. To provide context for the adulticidal resistance ratio of geraniol, similar experiments were performed using cypermethrin. Previously, we found the resistance ratio of adult females from this same PR strain of *Ae. aegypti* to cypermethrin was 84 [38]. As shown in Figure 2b, the 24 h LD_{50} of cypermethrin for the PS strain in the present study was 7.8×10^{-3} ng/mosquito (95% CI: 3.42×10^{-3}–1.76×10^{-2} ng/mosquito), whereas that for the PR strain was dramatically higher ($p < 0.0001$) at 3.55 ng/mosquito (95% CI: 2.42–5.10 × 4.81 ng/mosquito), indicating a resistance ratio of 457.

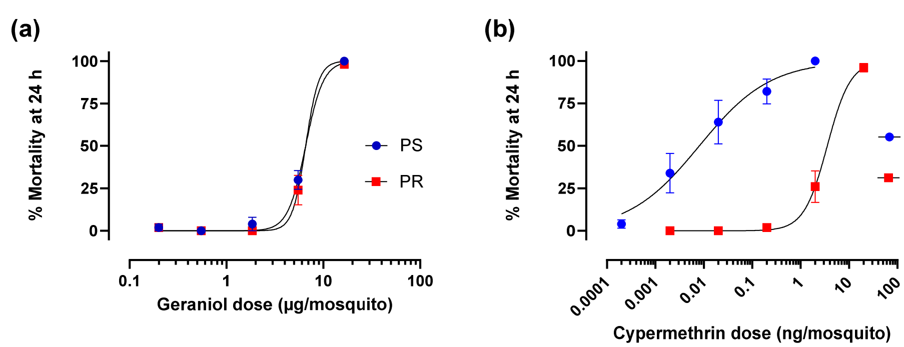

Figure 2. Dose-mortality curves for 24 h adulticidal activity of **(a)** geraniol and **(b)** cypermethrin against adult females of pyrethroid-susceptible (PS, blue) and pyrethroid-resistant (PR, red) *Ae. aegypti*. Values are means ± SEM based on 5 replicates per dose for both geraniol and cypermethrin for both strains. No mortality was observed in solvent controls.

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3.3. Geraniol and Pyrethrum Extract Spatial Repellency Against PS and PR Adult Females

Adult female *Ae. aegypti* from both PS and PR strains were spatially repelled by geraniol in a dose dependent manner (Figure 3a). The ED_{50} for the PS strain (0.74 µg/cm², 95% Cl: 0.58-0.95 µg/cm²) was lower ($p < 0.0001$) than that for the PR strain (1.90 µg/cm², 95% CI: 1.38–2.63 µg/cm²), indicating a repellency resistance ratio of 2.6.

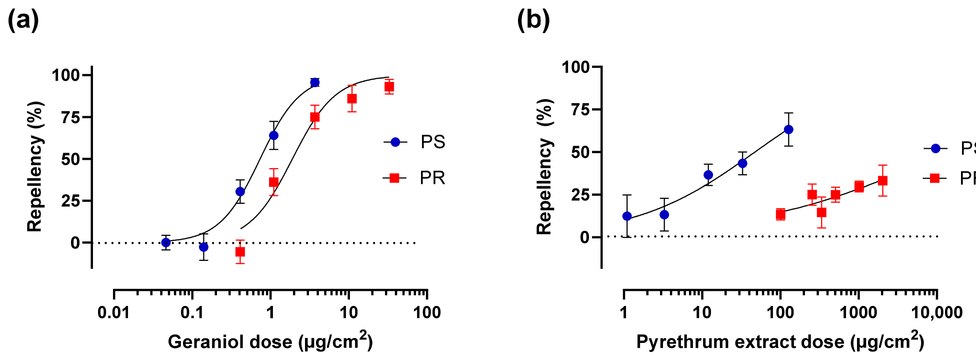

Figure 3. Dose-repellency (15 min) curves for (a) geraniol and (b) pyrethrum extract against adult females of pyrethroid-susceptible (PS, blue) and pyrethroid-resistant (PR, red) *Ae. aegypti.* For geraniol, values represent mean ± SEM based on 6-12 replicates per dose for PS and 6 replicates per dose for PR. For pyrethrum extract, values represent mean ± SEM based on 5 replicates per dose for PS and 5-8 replicates per dose for PR. Figure S1 shows the respective double-solvent controls for the geraniol and pyrethrum extract experiments.

To provide context for the repellency resistance ratio of geraniol, similar experiments were performed using pyrethrum extract. Previously, Yang et al. (2019) found the repellency resistance ratio of adult females from this same PR strain of *A. aegypti* to pyrethrum extract was 7.9 relative to the pyrethroid-susceptible Orlando strain of *A. aegypti*. As shown in Figure 3b, the ED_{50} of pyrethrum extract for the PS strain was 47.36 µg/cm² (95% CI: 23.82–140.7 µg/cm²). In contrast, pyrethrum extract showed weak dose-dependent spatial repellency against the PR strain that did not exceed 30%, indicating an ED_{50} that is beyond detectable limits. Based on the highest dose used in the present study (2,036.7 µg/cm²) we can assume the repellency resistance ratio is at least 132. Attempts to use higher doses of pyrethrum extract to drive higher repellency responses for both the PS and PR strains were unsuccessful due to vapor toxicity of the extract.

4. Discussion

The present study found that geraniol elicited concentration-dependent larvicidal activity against both PS and PR *Ae. aegypti*. The results for PS larvae were consistent with previous studies that found concentration-dependent larvicidal activity of geraniol against pyrethroid-susceptible strains of *Cx. pipiens*, *Cx. quinquefasciatus*, *An. stephensi*, *Ae. albopictus*, and *Ae. aegypti* [22,25,29,30]. Notably, larvae of the PR strain possessed modest cross-resistance (2.8 resistance ratio) to the toxicity of geraniol, but this was much lower than that for cypermethrin, a pyrethroid (435.3 resistance ratio). We also found that geraniol elicited dose-dependent adulticidal activity against both PS and PR *Ae. aegypti*. The results for PS adults were consistent with previous studies that found dose-dependent adulticidal activity in pyrethroid-susceptible strains of *Cx. pipiens*, *Ae. aegypti*, and *An. quadrimaculatus* [28,31]. In contrast to the larvicidal results, we found that adult females of the PR strain did not exhibit detectable cross-resistance to geraniol (1.1. resistance ratio) while they maintained strong resistance to cypermethrin (457 resistance ratio).

The PR strain of *Ae. aegypti* used in the present study (Puerto Rico) possesses target site resistance via *kdr* mutations in voltage-gated sodium channels and metabolic resistance likely via elevated expression of numerous CYP450 mRNAs and at least one GST mRNA [43]. Approximately 50% of

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the total resistance in the PR strain is attributed to enhanced CYP450 activity [43]. Thus, geraniol appears to bypass most of these resistance mechanisms in larvae and fully bypass them in adult females, suggesting that geraniol has strong potential as a toxin to control pyrethroid resistant populations of *Ae. aegypti*.

To understand why larvae, but not adult females, of the PR strain exhibited modest cross-resistance to geraniol will require further investigation into geraniol's mode of toxic action and how mosquitoes detoxify the compound. To date, biochemical targets of geraniol in mosquitoes remain unclear, but geraniol is known to inhibit acetylcholinesterase in beetles [44] and modulate octopaminergic signaling in neurons of cockroaches [45], suggesting neurological disruption as a potential mode of toxic action. A recent computational study suggested that geraniol may interact with voltage-gated sodium channels (VGSCs) in *Ae. aegypti* by binding to residues that are not implicated in *kdr* resistance [33]. Thus, if the toxicity of geraniol is associated with interactions with VGSCs, it may bind to the channels via a mechanism distinct from pyrethroids, which is consistent with the relatively modest cross-resistance to geraniol detected in larvae and adult females, respectively, compared to that for cypermethrin. The same computational study [33] also suggested that geraniol may interact with glutathione *S*-transferases (GSTs) and α-esterases in *Ae. aegypti*, which have both been implicated in metabolic resistance of mosquitoes to pesticides [46]. Thus, life stage-specific differences in the expression of these detoxification enzymes, as has been found in some mosquito species [42,47], could potentially lead to stage-specific differences in detoxification capacities for geraniol contributing to the different toxic resistance ratios in larvae vs. adult females. Future studies examining the impacts of synergists that inhibit CYP450s (e.g., PBO) or GSTs (e.g., diethyl maleate) on the toxicity of geraniol may help further elucidate the mechanisms of cross-resistance in larvae of the PR strain.

The present study also demonstrated that geraniol elicited dose-dependent spatial repellency against both PS and PR *Ae. aegypti*. The results in the PS strain were consistent with previous studies that found geraniol repelled pyrethroid susceptible strains of *Ae. aegypti* in both arm-in-cage and diffuser-based field assays [14,24]. However, our results conflict with a previous study in *Ae. aegypti* that found geraniol to be ineffective as a repellent when applied to ponies [32]. Notably, adult females of the PR strain possessed modest cross-resistance (2.6 resistance ratio) to the repellency of geraniol, but this was much lower than that for pyrethrum extract (>132 resistance ratio). Our repellency findings with geraniol are consistent with a previous study in the same PR strain of *Ae. aegypti* that found various degrees of repellency resistance to pyrethrum extract and pyrethroids (transfluthrin and metofluthrin), as well as other compounds, such as DEET, 2-undecanone, and IR3535, leading the investigators to propose that the pyrethroid resistance mechanisms in the PR strain offer broad resistance to a wide diversity of repellents [42]. It should be noted that the results for the PR strain of *Ae. aegypti* might not apply to pyrethroid-resistant strains of other mosquito species, because a pyrethroid resistant strain of *An. gambiae* did not exhibit cross-resistance to geraniol, whereas a carbamate-resistant strain of *An. gambiae* showed strong cross-resistance to geraniol [4]. Thus, the efficacies of various repellents against different insecticide resistant mosquito species should be confirmed on a species by species (and perhaps a strain by strain) basis.

Presumably, the strong repellency resistance to pyrethrum extract in the PR strain is due to *kdr* mutations that would weaken the interactions between natural pyrethrins and VGSCs; pyrethrins have been shown to drive the majority of the repellent activity associated with pyrethrum extract [48]. As mentioned above, although geraniol was predicted to interact with VGSCs in silico, it's binding mechanism is hypothesized to be distinct from that of pyrethroids or pyrethrins [33]. Thus, it is more likely that the modest repellency cross-resistance to geraniol is associated with the metabolic resistance mechanisms in the PR strain, which may also explain the broad repellent cross-resistance to DEET, 2-undecanone, and IR3535, which are not known to modulate VGSCs. Future studies examining the impacts of synergists that inhibit CYP450s (e.g., PBO) or GSTs (e.g., diethyl maleate) on the repellency of geraniol and other non-pyrethrin/pyrethroid compounds may help further elucidate the mechanisms of broad cross resistance to repellents in adult females of the PR

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strain. Nevertheless, given the relatively weak repellency cross-resistance to geraniol in the PR strain compared to that for pyrethrum extract found in the present study, our data suggest that geraniol bypasses most of the pyrethroid resistance mechanisms in *Ae. aegypti* and thereby has strong potential for use as a repellent against pyrethroid resistant populations of *Ae. aegypti*.

In conclusion, the results from our study support the notion that geraniol is a valuable natural product for developing novel mosquito control tools to combat pyrethroid resistance. However, it should be noted that the present study evaluated only one representative pyrethroid-susceptible and pyrethroid-resistant strain of *Ae. aegypti*. Thus, future studies in other species and resistant strains of mosquitoes are needed to confirm the broad applicability of these findings. Nevertheless, the promising results of the present study should motivate future studies to better characterize the biochemical and physiological modes of action of geraniol as a toxicant and repellent.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org, Figure S1: Double solvent controls (100% acetone on each side) for MART assays. For geraniol experiments, values represent mean ± SEM based on 12 replicates per dose for PS and 6 replicates per dose for PR. For pyrethrum extract experiments, values represent mean ± SEM based on 5 replicates per dose for PS and 8 replicates per dose for PR. None of the means were significantly different from zero as determined by a one-sample t-test (if data were normally distributed) or Wilcoxon signed-rank test (if data were not normally distributed). The results indicate that in the absence of geraniol or pyrethrum extract, mosquitoes from both strains show no preference for a side of the tube.

Author Contributions: Conceptualization, P.N.F. and P.M.P.; methodology, P.N.F. and P.M.P.; validation P.N.F..; formal analysis, P.N.F. and P.M.P.; investigation, P.N.F. and P.M.P.; resources, P.N.F. and P.M.P.; data curation, P.N.F. and P.M.P.; writing—original draft preparation, P.N.F. and P.M.P.; writing—review and editing, P.N.F., P.M.P.; visualization, P.N.F. and P.M.P.; supervision, P.M.P.; project administration, P.M.P; funding acquisition, P.N.F. and P.M.P. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are contained within the article or Supplementary Material.

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Conflicts of Interest: The authors declare no conflicts of interest.

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