

Anti-feeding efficacy of a human topical formulation of neem oil against *Aedes albopictus*

Gioia Bongiorno, Francesco Severini, Luigi Gradoni

Unit of Vector-borne Diseases and International Health, MIPI Department, Istituto Superiore di Sanità, Rome, Italy.

Background

Prominent botanical pesticides are those based on the neem tree extracts (*Azadirachta indica*, Meliaceae) whose seed kernels are rich in bioactive azadirachtin and other limonoids. Besides insecticidal effects, neem oil was shown to have anti-feeding activity against bloodsucking insects including anopheline and culicine mosquitoes, and phlebotomine sand flies. As with other natural products, neem oil has an excellent safety profile, however its persistence is affected by the rapid degradation of azadirachtin under natural conditions. Our objective was to determine the efficacy dose and duration of a commercial neem-oil product against the bites of the mosquito *Aedes albopictus*, a proven or potential vector of emerging Flaviviridae viruses in urban settings.

Methods

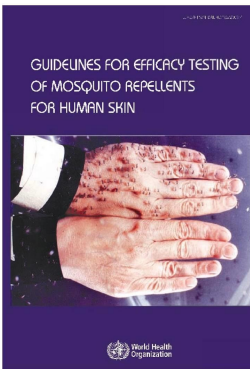


Figure 1. WHO Guidelines

A topical formulation of neem oil containing 2400 ppm azadirachtin (RP03[®] Human Care, Farmaneem, Italy) was employed at different dilutions on human volunteers, using laboratory-reared *Ae. albopictus*. Colonized *Culex pipiens* was also employed for comparison.

Assays were performed following WHO Guidelines for efficacy testing of mosquito repellents (Fig.1). Each of 2 volunteers performed from 6 to 9 replicate assays depending on experiment or species, using 40-200 caged female specimens. Assays consisted of 5 min exposure of the untreated hand (control) followed by 5 min exposure of the hand treated with 0.1 ml of serial product dilutions (in dose-response assays) or 0.25 ml of fixed dilutions (in duration assays). Insect probing attempts were recorded.

Results

Dose-response assays

In *Ae. albopictus*, the anti-feeding efficacy of neem oil increased significantly from 38.5% at 1% dilution to 99.5% at 15% dilution, with an ED₉₀ in the range of 6-10% (Fig.2).

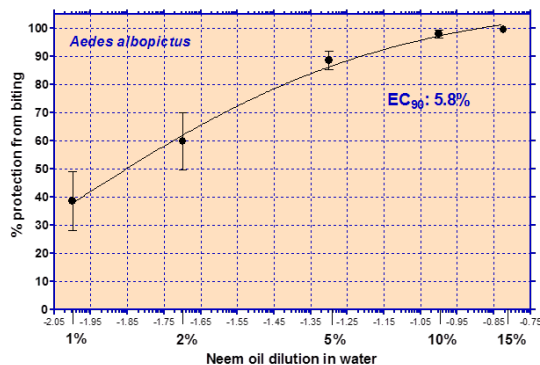


Figure 2. Antifeeding efficacy (mean SE) of serial dilutions of RP03[®] Human Care against *Ae. albopictus*

The product efficacy was found higher in assays using *C. pipiens*, for an ED₉₀ of less than 4% dilution (Fig.3).

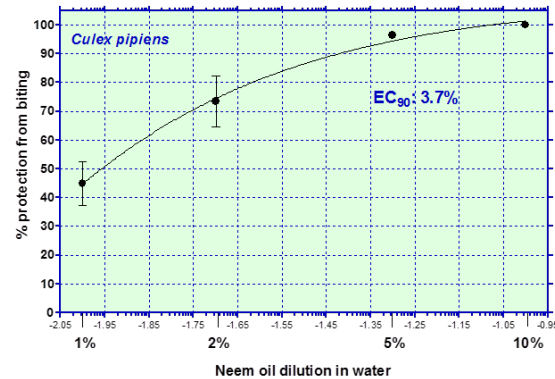


Figure 3. Antifeeding efficacy (mean SE) of serial dilutions of RP03[®] Human Care against *C. pipiens*

Duration assays

Because the antifeeding efficacy of neem-oil at dilutions of 6% and 10% did not differ significantly between them, we used one or the other dosage in duration assays, and the results from each of 9 *Ae. albopictus* replicates were analyzed together. The product was found to confer a mean protection rate of 92.3% at 5 min, which dropped to 36.0% (95% CI: 25.5-46.6%) at 3 hours from treatment, and further decreased thereafter. (Fig.4)

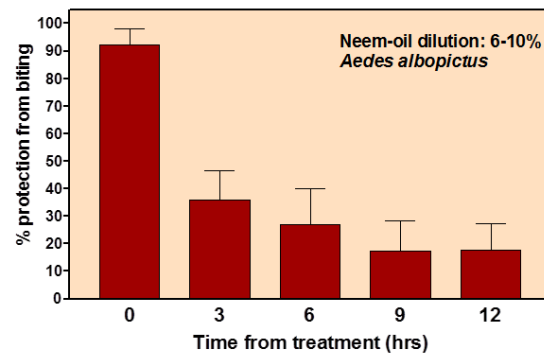


Figure 4. Antifeeding efficacy duration (mean 95% CI) of fixed doses of RP03[®] Human Care against *Ae. albopictus*

Conclusions

We conclude that topical neem oil can be highly effective for short-term exposures to *Ae. albopictus*, whereas long-term efficacy would require frequent applications which, however, are not contraindicated because of the excellent safety profile of the product.