



Studies on repellent activity of seed oils alone and in combination on mosquito, *Aedes aegypti*

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Abstract

The study was undertaken to investigate the relative repellency of *Pongamia pinnata* and *Azadirachta indica* seed oils on vector mosquito, *Aedes aegypti* under laboratory conditions. The repellents were formulated into 3 groups: seed oils, their mixture and combination of seed oils with three carrier oils viz. olive, mustard and coconut oil. Different formulations of each oil were tested at the concentrations of 1% and 5% on human baits. Efficiency was assessed, based on the total protection time; biting rate and percent protection provided by each formulation. Results showed that 5% formulation of the *Pongamia pinnata* and *Azadirachta indica* seed oils, mixed in 1:1 ratio exhibited highest percentage repellency of 85%, protection time of 300 min and bite rate of 6%. 5% concentration of *A. indica* and *P. pinnata* seed oil in mustard oil base offered 86.36% and 85% protection respectively with total protection time of 230 and 240 min respectively. The study confirms that *Azadirachta indica* and *Pongamia pinnata* have mosquito-repellent potential. When mixed in different ratios or with some carrier oil their efficacy increases 2-fold in some cases. These formulations are very promising for topical use (>5 hrs complete protection) and are comparable to the protection provided by advanced Odomos mosquito repellent cream available commercially and thus are recommended for field trial.

Key words

Aedes aegypti, Neem, *Pongamia*, Repellency, Synergism

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Introduction

Mosquito bite is the sole reason for transmission of dreadful diseases like malaria, dengue fever (DF), dengue hemorrhagic fever (DHF) and filariasis. Prevention of human-mosquito contact is thus, one of the main strategies to control or minimize the incidence of these diseases. A practical and economical means of preventing mosquito bites is the use of mosquito repellents. Repellent is defined as a chemical that causes insects to orientate away from the source (Miller, 2009). Many plants consist of volatile substances which repel insects. Several plant extracts have been studied as possible mosquito repellents and have demonstrated good efficacy against some mosquito species. Repellency of five plant essential oils--thyme, catnip, amyris, eucalyptus and cinnamon was tested against *Aedes albopictus*, *Aedes aegypti* and *Culex pipiens pallens* by Zhu *et al.* (2006). Plants like *Ocimum gratissimum*, *Clausena dentata*, *Eclipta prostrata*, *Tagetes erecta* have been reported to

possess strong repellent activity against mosquitoes (Rajkumar and Jebanesan, 2010; Oparaocha *et al.*, 2010; Elango *et al.*, 2010). Repellent activity of *Azadirachta indica* and *Eucalyptus* oil against *Culex quinquefasciatus* was studied by Mandal (2011) and it was observed that the oils showed excellent repellent action. Repellent activity of *Adansonia digitata* extracted with different solvents was studied against *Anopheles stephensi* by Krishnappa *et al.* (2012) and it was reported that methanol extract was the most effective and provided 100% protection for 210 minutes. Tennyson *et al.* (2012) studied the effect of combination of crude extracts of *Ageratum houstonianum* with coconut oil, on its repellent activity.

The common, commercially available mosquito repellents contain Deet (N, N-diethyl-3-methyl benzamide) which have shown excellent protection against mosquito bites (Thavara, 2001). Recently increasing efforts are being made to find and develop repellents derived from plant extracts (Tawatsin *et al.*,

2001; Trongtokit et al., 2005; Choochote et al., 2007). The aim of this study was to assess the repellency of seed oils and their combination against *A. aegypti*, under laboratory conditions using a screen cage method.

Materials and Methods

Laboratory bred *Aedes aegypti* mosquitoes were used in this study. Mosquitoes were reared at 26-28°C at 70-80% relative humidity; adults were fed with 10% sucrose. Prior to testing, 3 to 4 days old female mosquitoes were starved by providing them water for 12 hrs. The repellency of seed oils was assessed in the laboratory using human bait method (Barnard, 2005), following NIMR, New Delhi guidelines. Seed oils and their formulations with each other and with different carrier oils were used in the test (Table 1).

Since *Aedes aegypti* feed during daytime, the tests were performed between 0900 to 1700 hrs. For each test, 50 healthy *Aedes aegypti* female mosquitoes were placed in separate glass cages measuring 70 cm x 40 cm x 40 cm. All the adult females used in the experiments were reared in laboratory and hence were disease free. For each experiment new batch of adult females were taken. One side of the cage was covered with muslin cloth. A large opening was cut in the cloth which was tied by a string. This opening was used to introduce treated and control arms of human subjects.

Six human volunteers were selected and a written consent was taken from them before participating in the experiments. Before each test, the readiness of the mosquitoes to bite was confirmed by having subjects insert their untreated forearm into the test cage. Once subjects observed five mosquitoes landing on the untreated arm, they removed their arm from the cage. Each test repellent formulation (0.1 ml) was applied to the 25 cm² marked area of one forearm between elbow

and fingertips of each volunteer while unmarked area was covered by gloves. Separate experiments were conducted for each formulation. After application of each formulation, the solvent was allowed to evaporate for 30 min and subjects were instructed not to rub, touch or wet the treated arm. Control experiment was done by smearing the right arm of the subject with solvent for negative control and with advanced Odomas mosquito repellent cream for positive control.

Experiments to evaluate complete protection time were conducted according to the human bait method. The experiment was started after 30 min of application of oil. Treated and control arms were inserted in the cage simultaneously for 3 minutes. Observations were made for the first 3 min of every half an hour exposure. The test was continued until at least two bites occurred during the 3 min study period and followed by a confirmatory bite (second bite) in the following exposure period. The time between application of the repellent and the second successive bite was recorded as the protection time. For comparison, a percentage of mosquito bitings was calculated for each test using the formula of Amer and Mehlhorn (2006). The test was carried out 3 times per sample. Percentage protection provided by each plant and its various formulations was calculated by the formula of Venkatachalam and Jebanesan (2001).

Experiments to test the enhanced repellent effect, if any, on mixing *P. pinnata* and *A. indica* oils in 1:1, 2:1 and 1:2 ratios were also conducted. Different formulations of 1 and 5% of two oils were prepared by mixing with different carrier oils which were pure coconut, olive and mustard oils.

Statistical analysis: The mean protection time and percent bitings were used to compare the tested repellents. Difference in significance were analyzed by one-way analysis of variance (ANOVA).

Table 1 : Seed oils, their combination and seed oil + carrier oils and their concentrations used in the present study

| Seed oils | Combinations (Ratio) | Concentrations |
|--------------------------------------|---|-----------------------|
| Seed oils | | |
| <i>Pongamia pinnata</i> | Seed oil + Petroleum ether | 1 and 5% |
| <i>Azadirachta indica</i> | Seed oil + Petroleum ether | 1 and 5% |
| Combinations | | |
| Seed oil +Seed oil | | |
| <i>P. pinnata</i> + <i>A. indica</i> | Seed oil+Seed oil (1:1, 2:1, 1:2) | 1 and 5% |
| Seed oil + Carrier oil | | |
| <i>Pongamia pinnata</i> | Seed oil+Coconut oil (1:1, 2:1, 1:2) | 1 and 5% |
| | Seed oil+Olive oil (1:1, 2:1, 1:2) | 1 and 5% |
| | Seed oil+Mustard oil (1:1, 2:1, 1:2) | 1 and 5% |
| | Seed oil+Coconut oil (1:1, 2:1, 1:2) | 1 and 5% |
| | Seed oil+Olive oil (1:1, 2:1, 1:2) | 1 and 5% |
| <i>Azadirachta indica</i> | Seed oil+Mustard oil (1:1, 2:1, 1:2) | 1 and 5% |
| | Advanced Odomas repellent cream (Dabur brand) | 12mg cm ⁻² |
| Positive control | Petroleum ether solvent | 1 ml |
| Negative control | | |

Results and Discussion

Results showed a significant difference in repellency among oil formulations ($p < 0.05$). Seed oils of both plants showed high repellency against *Aedes aegypti* and the protection time of *Pongamia pinnata* and *Azadirachta indica* were 210 and 180 min respectively at 5% concentration. Bite rate and total protection afforded by both *P. pinnata* and *A. indica* oils were 6% and 85%. Results obtained in present investigation showed that seed oils of *Azadirachta indica* and *Pongamia pinnata* possess high repellency against *Aedes aegypti*.

Mosquitocidal and repellent activity of neem extract, oil, cake etc. have been reported earlier by several researchers (Sharma and Ansari, 1994; Caraballo, 2000). Neem oil (2%) mixed in coconut oil provided 96-100% protection from anophelines, 85% from *Aedes* and 37.5% from *Armigeres* mosquitoes whereas it showed wide range of efficacy, from 61-94% against *Culex* spp (Sharma *et al.* 1993; Sharma *et al.* 1995). Larvicidal and repellent action of *Pongamia pinnata* have been reported earlier (Deka *et al.*, 2010; Kulkarni and Aboli Lale, 2010).

The repellent effect of seed oils of *A. indica* and *P. pinnata* increased significantly on mixing. Combination of *A. indica* and *P. pinnata* seed oils showed best efficiency at 5% concentration in which the protection time was 300, 240 and 240 min, when mixed in 1:1, 2:1 and 1:2 ratios respectively, compared to 330 min in positive control with Advanced odomos repellent cream and min in negative control (Table 2). The results showed that repellent

efficiency of *A. indica* and *P. pinnata* improved when they were mixed in different ratios. Enhanced efficacy or synergistic effect of mixing various insecticides in different ratios have proved advantageous to control insects. *Pongamia* have been reported earlier to possess synergistic properties (Shanmugasundaram, 2008; Narasimhan *et al.* 1998; George and Vincent, 2005).

The synergistic effect of mixing *A. indica* and *P. pinnata* oils is well exhibited in the present study and the combination of seed oils was found to excel their individual's repellent effect against adult females of *Aedes aegypti*. This was probably due to the presence of different compounds in different plants that have different volatile properties. Protection time increased to 300 min when two oils were mixed in equal proportion compared to 180 min and 210 min for *P. pinnata* and *A. indica* oils, respectively. Similarly, 4 to 10-fold increase in the larvicidal action of neem and karanja oil cakes mixed in equal proportion was reported against vector mosquitoes (Shanmugasundaram, 2008).

Results for the repellency of seed oil mixed with carrier oil are shown in Tables 3 and 4. Protection provided by different combinations of *P. pinnata* oil with carrier oils was longer (240 min at 1:1 ratio of *P. pinnata* oil 5% + mustard oil) compared to *A. indica* oil (230 min at 1:1 ratio of *A. indica* oil 5% + mustard oil).

The percent protection and biting rate for all the tested formulations are shown in Tables 2, 3 and 4. The highest protection of 86.36% with biting rate of 6 % was observed when 5% of *A. indica* seed oil mixed with mustard oil in 1:1 ratio was

Table 2 : Values of total bites (numbers) and protection time of seed oils and their combinations against adult females of *Aedes aegypti*

| Seed oils | Doses (%) | Total bites (50 females tested) | | Protection time (min) | Percent biting | | Protection (%) |
|------------------------------------|-----------------------|---------------------------------|---------|--------------------------|----------------|---------|----------------|
| | | Control | Treated | | Control | Treated | |
| Seed oils | | | | | | | |
| <i>Pongamia pinnata</i> | 1% | 19 | 5 | 60 ± 0.00 ^h | 38 | 10 | 73.68 |
| | 5% | 20 | 3 | 180 ± 10.00 ^e | 40 | 6 | 85 |
| <i>Azadirachta indica</i> | 1% | 20 | 6 | 90 ± 0.00 ^g | 40 | 12 | 70 |
| | 5% | 20 | 3 | 210 ± 10.00 ^d | 40 | 6 | 85 |
| Combination of oils | | | | | | | |
| <i>A.indica</i> + <i>P.pinnata</i> | | | | | | | |
| Ratio: 1:1 | 1% | 21 | 4 | 120 ± 0.00 ^f | 42 | 8 | 80.95 |
| | 5% | 20 | 3 | 300 ± 0.00 ^b | 40 | 6 | 85 |
| 2:1 | 1% | 19 | 5 | 90 ± 0.00 ^g | 38 | 10 | 73.68 |
| | 5% | 20 | 5 | 240 ± 0.00 ^c | 40 | 10 | 75 |
| 1:2 | 1% | 20 | 6 | 90 ± 0.00 ^g | 40 | 12 | 70 |
| | 5% | 21 | 5 | 240 ± 0.00 ^c | 42 | 10 | 76.19 |
| Control | | | | | | | |
| Petroleum ether | 1ml | 20 | - | nil | 40 | nil | nil |
| Advanced Odomas cream | 12mg cm ⁻² | 20 | 3 | 330 ± 0.00 ^a | 40 | 6 | 85 |
| F value | | significant | | | | | |
| C.D.5% | | 7.46 | | | | | |

Values are mean of replicate ± SD; Means in each column followed by the different letter are significantly different ($p > 0.05$, by one-way ANOVA)

Table 3 : Values of total bites (numbers) and protection time of combination of *Azadirachta indica* seed oil with carrier oils against females of *Aedes aegypti*

| Combination of oils | Doses (%) | Ratio | Total bites (50 females tested) | | Protection time (min) | Percent biting | | Protection (%) |
|---------------------------------|-----------|-------|---------------------------------|-------------|-------------------------|----------------|---------|----------------|
| | | | Control | Treated | | Control | Treated | |
| Azadirachta indica +Olive oil | 1% | 1:1 | 19 | 5 | 60±0.00 ^d | 38 | 10 | 73.68 |
| | | 2:1 | 20 | 6 | 60±0.00 ^d | 40 | 12 | 70 |
| | | 1:2 | 20 | 6 | 50±10.00 ^{de} | 40 | 12 | 70 |
| | 5% | 1:1 | 20 | 4 | 200±10.00 ^b | 40 | 8 | 80 |
| | | 2:1 | 21 | 4 | 160±10.00 ^{bc} | 42 | 8 | 80.95 |
| | | 1:2 | 19 | 5 | 180±0.00 ^b | 38 | 10 | 73.68 |
| Azadirachta indica +Coconut oil | 1% | 1:1 | 20 | 6 | 60±0.00 ^d | 40 | 12 | 70 |
| | | 2:1 | 18 | 5 | 50±10.00 ^{de} | 36 | 10 | 72.22 |
| | | 1:2 | 20 | 6 | 60±0.00 ^d | 40 | 12 | 70 |
| | 5% | 1:1 | 21 | 4 | 200±10.00 ^b | 42 | 8 | 80.95 |
| | | 2:1 | 20 | 5 | 180±0.00 ^b | 40 | 10 | 75 |
| | | 1:2 | 21 | 5 | 170±10.00 ^{bc} | 42 | 10 | 80 |
| Azadirachta indica +Mustard oil | 1% | 1:1 | 20 | 6 | 80±10.00 ^d | 40 | 12 | 70 |
| | | 2:1 | 20 | 7 | 60±0.00 ^d | 40 | 14 | 65 |
| | | 1:2 | 21 | 6 | 60±0.00 ^d | 42 | 12 | 71.42 |
| | 5% | 1:1 | 22 | 3 | 230±10.00 ^a | 44 | 6 | 86.36 |
| | | 2:1 | 21 | 5 | 170±10.00 ^{bc} | 42 | 10 | 76.19 |
| | | 1:2 | 20 | 4 | 170±10.00 ^{bc} | 40 | 8 | 80 |
| F value | | | | Significant | | | | |
| C.D.5% | | | | 21.37 | | | | |

Values are mean of replicate ±SD; Means in each column followed by the different letter are significantly different (p>0.05, by one- way ANOVA)

Table 4 : Values of total bites (number) and protection time of combination of *Pongamia pinnata* seed oil with carrier oils against females of *Aedes aegypti*

| Combination of oils | Doses (%) | Ratio | Total bites (50 females tested) | | Protection time (min) | Percent biting | | Protection (%) |
|--------------------------------------|-----------|-------|---------------------------------|---------|--------------------------|----------------|---------|----------------|
| | | | Control | Treated | | Control | Treated | |
| <i>Pongamia pinnata</i> + Olive oil | 1% | 1 : 1 | 20 | 7 | 90±0.00 ^d | 40 | 14 | 65 |
| | | 2 : 1 | 21 | 6 | 80±10.00 ^d | 42 | 12 | 71.42 |
| | | 1 : 2 | 21 | 7 | 80±10.00 ^d | 42 | 14 | 66.66 |
| | 5% | 1 : 1 | 20 | 4 | 230±10.00 ^a | 40 | 8 | 80 |
| | | 2 : 1 | 21 | 6 | 210±0.00 ^{ab} | 42 | 12 | 71.42 |
| | | 1 : 2 | 20 | 6 | 210±0.00 ^{ab} | 40 | 12 | 70 |
| <i>Pongamia pinnata</i> +Coconut oil | 1% | 1 : 1 | 20 | 6 | 80±10.00 ^d | 40 | 12 | 70 |
| | | 2 : 1 | 19 | 6 | 80±10.00 ^d | 38 | 12 | 68.42 |
| | | 1 : 2 | 19 | 6 | 90±0.00 ^d | 38 | 12 | 68.42 |
| | 5% | 1 : 1 | 21 | 4 | 230±10.00 ^a | 42 | 8 | 80.95 |
| | | 2 : 1 | 20 | 5 | 210±0.00 ^{ab} | 40 | 10 | 75 |
| | | 1 : 2 | 21 | 6 | 210±0.00 ^{ab} | 42 | 12 | 71.42 |
| <i>Pongamia pinnata</i> +Mustard oil | 1% | 1 : 1 | 18 | 6 | 110±10.00 ^d | 36 | 12 | 66.67 |
| | | 2 : 1 | 19 | 6 | 90±0.00 ^d | 38 | 12 | 68.42 |
| | | 1 : 2 | 19 | 5 | 80±10.00 ^d | 38 | 10 | 73.68 |
| | 5% | 1 : 1 | 20 | 3 | 240±0.00 ^a | 40 | 6 | 85 |
| | | 2 : 1 | 20 | 3 | 200±10.00 ^{abc} | 40 | 6 | 85 |
| | | 1 : 2 | 18 | 4 | 200±10.00 ^{abc} | 36 | 8 | 77.78 |
| F value | | | | | Significant | | | |
| C.D.5% | | | | | 21.37 | | | |

Values are mean of replicate ±SD; Means in each column followed by the different letter are significantly different (p>0.05, by one- way ANOVA)

applied on skin of the subject. Good efficiency for adequate times against *A. aegypti* was exhibited by *P. pinnata* (5%) (for 180 minutes with 6% bite rate and 85% protection), *A. indica* (5%) (for 210 min with 6 % bite rate and 85% protection), *A.indica* + *P.pinnata* (5%, 1:1seed oils) (for 300 min with a 6% bite rate and 85% protection) and *P.pinnata* (5%) + mustard oil (1:1) (for 240 min with 6% bite rate and 85% protection), comparable to percent protection and biting seen on applying Odomos repellent cream (for 330 min with 6% bite rate and 85% protection). Highest biting (10-14%), lower protection time (>110 min) and lower percent protection (>73.68) was observed at 1% of *P.pinnata* and *A.indica* oils in different combinations with three carrier oils.

Several factors (type of repellents, mode of application, local weather conditions volatile nature of repellent etc.) affect the efficacy and duration of repellent chemicals. Plant extracts and essential oils with high volatility commonly act on mosquitoes in the vapor phase (Browne,1997; Zhu *et al.*, 2001) which is effective for a relatively short period (Rozendaal,1997; Barnard, 2000). Plant oils when formulated with a base or fixative material, such as vanillin, liquid paraffin, mustard and coconut oil have shown improved repellency (Das and Ansari, 2003; Oyedele *et al.*, 2002). This improvement in repellency on mixing repellent oil with base oil is probably due to the formulation formed could fix the aromatic constituents of oil onto skin for an appropriate time.

This study evidently demonstrated better protection against *A. aegypti* mosquitoes from *A. indica* and *P. pinnata* seed oils after addition of carrier oils such as olive, coconut and mustard oil. The formulation of *P. pinnata* and *A. indica* seed oils both at 5 % (with mustard oil base) concentrations, exhibited excellent repellency against *Aedes aegypti* bites. Recently, various oils, creams, polymer mixtures, or microcapsules based formulations which enable slow and controlled release of repellent vapours, resulted in an increase of repellency duration (Gupta and Rutledge, 1991).

From the present investigation it is evident that although *P.pinnata* and *A. indica* seed oils were effective individually, their repellent effect in terms of protection time, biting rate and percent protection could be increased significantly on mixing in different ratios. The mosquito bite-deterrent effect of the two oils mixed in 1:1 ratio was enhanced significantly (5 hrs complete protection from mosquito bite).

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