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# Comparative efficacy of *Annona squamosa* Linn. and *Pongamia glabra* Vent. to *Azadirachta indica* A. Juss against mosquitoes

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While most people consider mosquitoes as an annoyance, these tiny assassins have the potential and lethal capacity to kill more than a million victims a year around the world[1]. Mosquito borne diseases such as malaria, filariasis, dengue, yellow fever and encephalitis are continuing to be major health problems for the people[2]. Malaria alone kills three million people annually, including one child every 30 sec [3,4]. Vector control technologies such as 'source reduction', application of oils and botanical preparations were emphasised in the second quarter of the last century[5]. But with the advent of DDT towards the end of the second quarter, an unprecedented flurry ensued for finding synthetic compounds. These compounds were very active against mosquito larvae but were highly toxic to non-targeted organisms. Pesticide exposure among humans has been linked to immune dysfunction, various forms of cancer and birth defects[6,7]. It is, therefore, necessary to identify a safe, eco-friendly alternate source of larvicide in order to reduce mosquito menace.

Over two thousand species of plants are known to possess insecticidal activity[8]. Some of the plants that have been tested against mosquito larvae in India are *Cleome viscosa*, *Ocimum basilicum*, *Vitex negundo*, *Delonix regia* and *Oligochaeta ramosa*, *Azadirachta indica*, *Quassia amara*, *Anacardium occidentale* and *Thevetia neriifolia*[9–11]. Natural products are preferred because of their innate biodegradability[12]. *Annona squamosa* was evidenced to be larvicidal against larvae and pupae of *Culex quinquefasciatus*[13]. 'Karanja oil' and 'neem oil', were also proven to be potential larvicides against *Culex*[14]. Besides the karanja extract from the trees of *Pongamia* has been suggested as a new synergist[15,16]. Neem has been acknowledged as a prominent biopesticide in recent years. However, as the mosquito larvicidal and growth regulating activity of neem has been widely established, it has also been emphasised that if used indiscriminately in blanket sprays, they may induce resistance in the pests and can be rendered ineffective within a few years[17]. Thus, the finding of potent botanical pesticides is inevitable and is the need of the hour. Hence, this study to compare the individual and combined efficacy of the extracts of *A. squamosa* and *Pongamia glabra* to *Az. indica* was undertaken.

The larvae of *Culex quinquefasciatus* collected from the field were maintained at 70–85% relative humidity, 27± 2°C temperature and 14:10 light and dark photoperiod cycle. The larvae were fed on a powdered mixture of dog biscuits and yeast tablets in the ratio of 3:1. The emerged adults were fed with rabbit blood and with 10% glucose solution.

Seeds of selected plants were collected from in and around Chennai, shade dried at room temperature, powdered coarsely and extracted with petroleum ether for eight hours in the soxhlet apparatus (300 ml of petroleum ether for 100 g)[18]. The weighed quantity of the plant material (300 g) was reduced to a viscous dark green residue (7.5 g). The crude extracts were further concentrated to pastes.

Stock solution (1000 ppm) was prepared by dissolving 250 mg of the crude extract in 5 ml of acetone and making it to 250 ml by mixing distilled water in a standard flask. All the test solutions were made by diluting the known volume of stock solution of the extract with water[19]. The synergistic factor (SF) was calculated using the formula[20].

$$SF = \frac{LC_{50} \text{ value of the insecticide alone}}{LC_{50} \text{ value of the insecticide with the assumed synergist}}$$

(Values of SF > 1 indicate synergism and SF < 1 indicate antagonism).

The test solution of 250 ml at various concentrations was made in 500 ml beaker by adding known volume of stock solution. Twenty-five fourth instar larvae were seeded in the test suspension. Four replicates were set up at each concentration[13]. The control was also run simultaneously. The corrected mortality was calculated by using Abbott's formula[21]. The mortality was counted after 24 h and the $LC_{50}$ values were calculated according to Probit analysis[22].

The field collected fourth instar larvae (FCL) and the laboratory colonised larvae (LCL) of *Cx. quinquefasciatus* were subjected to various concentrations of the extract of *A. squamosa* (A 100%), *P. glabra* (P 100%) independently and *A. squamosa* and *P. glabra* combined in the ratios of A 75% : P 25%, A 50% : P 50% and A 25% : P 75% respectively. The FCL and LCL were also subjected to the extracts of *Az. indica* (N 100%) for subsequent comparison of their efficacy. The *Annona–Pongamia* combination extract with the maximum larvicidal potential from the earlier test was compared with the extract of neem (N 100%) to study their effect on the LCL of *An. stephensi* and *Aedes aegypti* as well.

**Table 1. Bioassay studies on the early IV instar field collected larvae (FCL) and laboratory colonised larvae (LCL) of *Cx. quinquefasciatus* against petroleum ether seed extracts of selected plants**

| S.No. | Name of extract | Combination of extract | Field collected larvae | | | | Laboratory colonised larvae | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $LC_{50}$ (ppm) | Fiducial limits | | Regression equation | $LC_{50}$ (ppm) | Fiducial limits | | Regression equation |
| | | | | Upper | Lower | | | Upper | Lower | |
| 1. | *Annona squamosa (A)* | A 100% | 674.41 | 798.84 | 549.98 | Y = – 0.909+ 1.735 log× | 436.110 | 506.56 | 365.66 | Y = 3.108 + 1.882 log × |
| 2. | *Pongamia glabra (P)* | P 100% | 282.57 | 309.92 | 255.22 | Y = –0.942 + 2.424 log × | 138.030 | 150.09 | 125.97 | Y = –1.499 + 3.037 log × |
| 3. | *A. squamosa & P. glabra* | A 75% : P 25% | 233.36 | 255.68 | 211.04 | Y = –1.415 + 2.709 log × | 82.770 | 91.49 | 74.05 | Y = –1.586 + 3.434 log × |
| 4. | *A. squamosa & P. glabra* | A50% : P 50% | 161.29 | 178.22 | 144.36 | Y = –1.359 + 2.88 log × | 28.804 | 31.301 | 26.307 | Y = 0.629 + 2.995 log × |
| 5. | *A. squamosa & P. glabra* | A 25% : P 75% | 257.34 | 281.00 | 233.68 | Y = –1.471 + 2.684 log × | 112.104 | 122.35 | 101.86 | Y = –1.758 + 3.297 log × |
| 6. | *Azadirachta indica (N)* | N 100% | 176.91 | 195.15 | 158.66 | Y = –1.174 + 2.747 log × | 45.120 | 49.43 | 40.81 | Y = 1.374 + 2.192 log × |

Table 1 reveals the results from 24 h bioassay studies against the petroleum ether extracts of the seeds of 'A 100%, P 100%' and their combinations. The 'P 100%' extract showed a greater larvicidal effect over the 'A 100%' independent extract. Among their combined extracts the 'A 50% : P 50%' extract was found to be more toxic than the other combinations. The most effective extract, 'A 50% : P 50%' was found to be significantly more effective than the *Az. indica* (N 100%) extract. This combination, revealed maximum synergism, the *Pongamia* extract being considered as the synergist in the present study (Table 2). Table 3 reveals the extracts to be toxic to the larvae of the three prominent vectors, namely *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The larvae of *Ae. aegypti* were most susceptible followed by *Cx. quinquefasciatus* and *An. stephensi* respectively to both the extracts.

Several workers have suggested various larvicidal plant species in the control of mosquitoes. In the acute toxicity tests against the fourth instar FCL and LCL of *Cx. quinquefasciatus* the larval mortality increased with increased concentrations of the extract. Similar observations were made by several workers[23,13]. The FCL were apparently better adapted to adjust to stress variations in the environment and hence required a higher concentration of the extracts to bring about the required mortality in all the cases. The possible reason for the death of the larvae subjected to the extract was attributed to the presence of feeding inhibiting substances in the extract. On the exposure to high concentrations, the larval body retraction accompanied with a sluggish surface behaviour was observed. Combined effect or synergistic effect of various control agents have proved very advantageous in the con-

**Table 2. SF values of combined extracts of *Annona* and *Pongamia* against the fourth instar laboratory colonised larvae (LCL) of *Cx. quinquefasciatus***

| S.No. | Name of extract | Combination of extract | $LC_{50}$ (ppm) | Synergistic factor (SF) | Effect |
|---|---|---|---|---|---|
| 1. | *Annona squamosa (A)* | A 100% | 4.361 | — | — |
| 2. | *Pongamia glabra (P)* | P 100% | 1.380 | — | — |
| 3. | *A. squamosa & P. glabra* | A 75% : P 25% | 0.828 | 5.3 | Synergism |
| 4. | *A. squamosa & P. glabra* | A 50% : P 50% | 0.288 | 15.1 | Synergism |
| 5. | *A. squamosa & P. glabra* | A 25% : P 75% | 1.120 | 3.9 | Synergism |

**Table 3. Bioassay studies on the early fourth instar larvae of different mosquito species against petroleum ether seed extracts of selected plants**

| S.No. | Name of extract | Combination of extract | $LC_{50}$ (ppm) | Fiducial limits | | Regression equation |
|---|---|---|---|---|---|---|
| | | | | Upper | Lower | |
| ***An. stephensi*** | | | | | | |
| 1. | *A. squamosa (A) & P. glabra (P)* | A 50% : P 50% | 59.75 | 66.59 | 52.91 | Y= – 2.277 + 4.097 log × |
| 2. | *Az. indica (N)* | N 100% | 75.65 | 83.22 | 68.08 | Y= – 2.355 + 3.915 log × |
| ***Ae. aegypti*** | | | | | | |
| 1. | *A. squamosa (A) & P. glabra (P)* | A 50% : P 50% | 27.288 | 29.65 | 24.93 | Y = 0.553 + 3.097 log× |
| 2. | *Az. indica (N)* | N 100% | 39.388 | 42.88 | 35.80 | Y = 1.074 + 2.462 log× |

trol of various pests[24,25]. The *Pongamia* extract has been considered as a good synergist and hence has been combined with several pest control agents in the control of various pests[26,27]. In the present study likewise the *Pongamia* extract has acted as a powerful synergist with *A. squamosa*. Individual plant extracts are active only at high concentration, which makes them uneconomical for field use.

Besides, it has been reported as the continuous use of synthetic insecticides caused insect pests and vectors to develop resistance in due course of time, it is often more economical to use synergists. This synergistic mixture can be incorporated into mosquito control programmes as well, so as to avoid indiscriminate use of neem in blanket sprays. Such strategies will minimise the problem of induction of resistance in the pest population and will apparently continue to render the extracts 'effective' for many years as pest control agents.

From the studies on mosquito species susceptibility to different extracts, it was concluded that the various species of mosquitoes showed differential susceptibility to different extracts as reported earlier[28]. Neverthe less, the synergistic mixture proved to be toxic to the larvae of *An. stephensi* and *Ae. aegypti* as well.

Neem although fully acknowledged for its pesticidal potential has not been recommended to be used indiscriminately in blanket sprays. Thus the search for an alternative effective biopesticide is inevitable. The present study has attempted to suggest a better alternative or an effective substitute in the form of a synergistic mixture of *A. squamosa* and *P. glabra* extracts, to the *Az. indica* extract which has been widely acknowledged and currently available as a prominent biopesticide.

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