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Toxicity of *Acalypha indica* (Euphorbiaceae) and *Achyranthes aspera* (Amaranthaceae) leaf extracts to *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT

Alternative control strategies for the dengue vector *Aedes aegypti* L. (Diptera: Culicidae) include botanical insecticides. They are believed to pose little threat to the environment or to human health and may provide practical substitutes for synthetic insecticides. In this study, we determined the biological activities of methanol extracts of *Acalypha indica* L. (Euphorbiaceae) and *Achyranthes aspera* L (Amaranthaceae) leaves individually and in combination as botanical insecticides against *Ae. aegypti*. Based on LC_{50} values for 4th instar *Ae. aegypti*, the combined extracts showed the strongest larvicidal activity (277 ppm). *A. aspera* and *A. indica* extracts individually gave similar results (409 and 420 ppm, respectively). Respective LC_{50} values for pupae were 326 ppm, 456 ppm, and 467 ppm. In studies of smoke toxicity, 64% of females exposed to negative control smoke (no extract) blood fed on chicken, whereas 17% blood fed when exposed to smoke containing *A. aspera* extract and to positive control smoke (0.2% d-allethrin). In the field, treatment of water storage tanks ($\approx$0.5 m^3) with combined plant extract reduced larval and pupal populations by 97% and 81%, respectively, after 5 days. Given the results of this study, further evaluation of the combined (*A. indica* + *A. aspera*) extract as a mosquito larvicide is warranted. Mosquito coils with *A. aspera* extract also show promise as a practical and potentially economical means for mitigating mosquito blood feeding.

Introduction

The container breeding mosquito, *Aedes aegypti* L. thrives in urban and peridomestic environments where it transmits the dengue virus to humans (Gubler, 1998). More than 50 million people are at risk of dengue virus exposure worldwide. Annually, there are 2 million infections, 500,000 cases of dengue hemorrhagic fever, and 12,000 deaths (Guha-Sapir and Schimmer, 2005).

Conventional control of *Ae. aegypti* is based on treatment of water containers with larvicides and on the use of adulticides (Gubler and Clark, 1995; Gubler, 2004). Resistance of *Ae. aegypti* to organochlorine and organophosphate insecticides, including temephos, is widespread (WHO, 1996; Suárez et al., 1998; Rodriguez et al., 2007). Community-based environmental sanitation and biological control strategies have been more recently incorporated (Ocampo et al., 2009; Kay et al., 2010). These focus educational and social efforts on the elimination of mosquito production sites within the community and may be combined with the use of mosquito predators and pathogens, although these strategies are difficult to implement effectively in the short-term.

Alternative control technologies for *Ae. aegypti* include the use of plant-based larvicides and adulticides. These botanical insecticides are believed to pose little threat to the environment or to human health and may provide a practical substitute for synthetic insecticides (Isman, 2006). To date, a number of phytochemicals with biological activity against immature and adult mosquitoes have been described (Murugan and Jeyabalan, 1999; Pelah et al., 2002; Murugan et al., 2007; Rahuman and Vekatesan, 2008; Bagavan et al., 2008; Rahuman et al., 2009; Zahir et al., 2009; Govindarajan, 2010).

Acalypha indica L. (Euphorbiaceae) (common name: Indian copperleaf) is an annual herb found throughout the Indian peninsula and in southern China, tropical and South Africa, Sri Lanka, Pakistan, and Yemen. It is used by healers for the treatment of malarial fever, dysentery, and diabetes, and it possesses snake venom neutralizing properties (Girach and Khan, 1992; Liersch, 1992; Shirwaikar et al., 2004; Ragupathy and Newmaster, 2009). Cyanogenesis of *A. indica* has long been known (Hungeling et al., 2009). Leaf extracts of this species have been studied as ovicides, larvicides, and oviposition attractants for *Anopheles stephensi* Liston (Govindarajan et al., 2008).

Achyranthes aspera L. (Amaranthaceae) (common name: devil's horsewhip) is a widely naturalized herbaceous plant species native to Africa and Asia. It is used for the reclamation of wastelands, and the seeds and leaves are used for human consumption and in religious

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ceremonies in India (Oudhia, 2004; Ragupathy and Newmaster, 2009). The leaf extract is prothyroidic and antiperoxidative in rats (Tahiliani and Kar, 2000). Saponins from the ethyl acetate extract of *A. aspera* leaves have been tested as larvicides against *Ae. aegypti* and *Culex quinquefasciatus* Say (Bagavan et al., 2008), and extracts of the leaves, the flowers, and the seeds of *A. aspera* have biological activity against *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini 1887) (Acari: Ixodidae) (Zahir et al., 2009) and against the 4th instars of *Anopheles subpictus* Grassi and *Culex tritaeniorhynchus* Giles mosquito larvae (Bagavan et al., 2008).

In this study, we determined the biological activity of extracts of the leaves of *A. indica* and *A. aspera* individually and in combination as botanical insecticides against *Ae. aegypti*. In the laboratory, we tested the effectiveness of each extract as a larvicide and as a pupicide and determined smoke toxicity on survival and blood feeding in female mosquitoes. We also performed a field test to assess the effectiveness of each extract for reducing immature mosquito populations in water containers in local villages.

Materials and methods

Mosquito culture

Ae. aegypti were collected as eggs from wild populations in and around Coimbatore. Rearing conditions for all mosquitoes were 27 ± 2 °C, 75–85% RH, and a L14: D10 photoperiod. Larvae were reared in 2 l of water in enamel trays (30 cm long × 25 cm wide × 6 cm deep) where they were provided a daily food mix comprising 3 parts brewers yeast and 1 part dog biscuit. Pupae were placed into screened cages (23 cm long × 23 cm wide × 23 cm deep). When adults emerged after 24 h, they were transferred to glass cages (30 cm long × 30 cm wide × 30 cm deep) and provided with 10% sucrose solution (in water) via cotton wick. Five days after emergence, female mosquitoes were allowed access to a restrained 1 week-old chick for blood feeding.

Plant specimens and preparation of extract

Specimens of *Acalypha indica* L. (Euphorbiaceae) and *Achyranthes aspera* L. (Amaranthaceae) were collected near the Bharathiar University campus. These were identified to species by personnel at the Botanical Survey of India (BSI) in Coimbatore and voucher specimens deposited with BSI.

In the laboratory, leaves of *A. indica* and *A. aspera* were rinsed with tap water and shade-dried at room temperature (27 °C). These were pulverized into a powder (50–100 μm dia) using an electric blender. One kg of powder was extracted using 3 l of methanol in a Soxhlet apparatus for 8 h (Vogel, 1978). The extract was then concentrated in a rotary vacuum evaporator. One gram of this product was dissolved in acetone (100 ml final volume) to make a 1% (10,000 ppm) stock solution of extract.

Larval and pupal bioassays

Bioassays were performed in a 500 ml paper cup containing 250 ml (final volume) of water and one of five treatment solutions achieved by the addition of 2, 4, 6, 8, or 10 ml of leaf extract stock solution to the water in the cup. The resulting concentrations were 80, 160, 240, 320, and 400 ppm of plant extract, respectively. The controls contained an appropriate quantity of acetone in lieu of plant extract. For each assay, 25 larvae or 20 pupae were placed in the (as yet) untreated water (along with a small quantity of food in the case of the larvae) and allowed to equilibrate for 1 h. Larval or pupal responses were observed 24 h after the addition of plant extract to the test unit. Each life stage was scored as dead when unreactive to prodding with a small wooden dowel. A single bioassay series for each life stage included the five plant extract concentrations specified above plus a control. The life stages tested were instar 1 (at 1–2 days after hatching) instars 2 through 4 (24–36 h after molting) and the pupa (24–36 h after pupation). Each series was replicated three times for each instar ($n = 72$) and for pupae ($n = 18$). Environmental conditions during larval and pupal bioassays were the same as for general mosquito rearing.

Smoke toxicity assay

Powdered plant extract was used to make mosquito coils for burning to produce smoke for toxicity assays. The coils were prepared using the technique described by Saini et al. (1986). Each coil included 5 g of extracted plant material (2.5 g of *A. indica* extract and 2.5 g of *A. aspera*), 2 g of saw-dust as a binding agent, 2 g of coconut shell charcoal for fuel, and sufficient distilled water for all ingredients to form a semisolid paste. The material was mixed thoroughly by hand and then shaped into a strand of consistent diameter (0.6 cm) using a press, coiled, and allowed to dry outdoors in the shade for 48 h. Coils used for the negative control were prepared in the same manner as the treatment coils but without the plant ingredients. Positive control coils (Mortein 8 Hour Green Coil®; PT Reckitt Benckiser Indonesia, Artha Graha Building, Jl.Jend. Sudirman Kav. 52-53 Jakarta 12190, Indonesia) contained 0.2% d-allethrin.

Smoke toxicity tests were performed inside a 60 cm^3 cage (2.16×10^5 cm^3) with a glass front, wooden sides, and muslin cloth covered top using the technique described by Murugan et al. (2007). A door (30 cm wide × 30 cm high) on one side of the cage enabled access to the cage interior. In a subjective attempt to maintain consistency in the quantity and quality of smoke produced by each coil, we burned 1 coil continuously in the test cage every 12 h over a 144 h period (i.e., 12 coils were burned over 6 d). At approx. the same time on each of the last 5 d of the 6 d period (day 1 was used to achieve and maintain a consistent smoke density), 50, nulliparous females (3–4 days-old) were introduced into the smoke-filled cage and the cage closed with the mosquitoes inside for 30 min. At the same time, a restrained chick was made available to the mosquitoes in the cage for blood feeding. After 30 min, all mosquitoes were removed from the cage and classified as either dead or alive and as blood fed or not depending on the presence or absence of blood in the abdomen.

Tests of positive and negative smoke controls were made in the same manner. For each treatment, each day of the 5 d test was considered one of 5 replicates for that treatment.

Field trial

A field trial to determine the effects of *A. indica* and *A. aspera* extracts on *Ae. aegypti* larval and pupal populations in water storage tanks was made outdoors in three villages near Coimbatore (Vadavalli [treatment: *A. indica* extract], Kalveerampalayam [treatment: *A. aspera* extract], and Ponmanapalayam [treatment: *A. indica* + *A. aspera* extract]). In each test, a total of 100 g of extract (50 g each for the *A. indica* + *A. aspera* test) dispersed with 0.5 g of 0.05% Teepol® emulsifying agent in 1 l (final volume) of water. The solution was applied by hand pump sprayer to the surface of the approximately 0.5 m^3 of water contained in each tank until a concentration of approx. 200 ppm was achieved. A negative control was not used; however, an index of the numbers of immature mosquitoes in each container was determined before treatment by counting the total number of larvae and pupae captured in each of five ≈ 250 ml samples, using a 500 ml dipper for each sample. An index of the post-treatment mosquito populations was determined 24 h later in the same manner and at 24 h intervals thereafter for a total of 120 h (5 days).

Statistical analysis

Probit analysis (Finney, 1971) was used to determine the dose–response relationship for *A. indica*, *A. aspera*, and *A. indica* + *A. aspera* extracts against *Ae. aegypti* larvae and pupae. Responses were corrected for control mortality prior to data analysis using Abbott's formula (Abbott, 1925). Percent mortality and blood feeding of females in smoke exposure tests, along with change in larval and pupal numbers in field tests were analyzed using ANOVA with means separation using Tukey's Honestly Significant Difference (HSD) test in the former case and Dunnett's one-tailed *t* test in the latter case to identify any significantly lowered post-treatment means (SAS, 2003). For the results of the field trial, each count datum was transformed to $\log_{10}(n+1)$ before ANOVA. The significance level used in all statistical tests was $P = 0.05$.

Results

Larvicidal and pupacidal bioassays

The median lethal concentration (LC_{50}) of the combined extract for 4th instar *Ae. aegypti* (277 ppm) was two-thirds that for either *A. indica* (420 ppm) or *A. aspera* (409 ppm) (Table 1). Similarly, the LC_{50} for pupae using the combined extract (326 ppm) was 30% lower than that for either the *A. indica* (467 ppm) and *A. aspera* (456 ppm) extracts. Fitted slopes (Table 1) for all extracts indicate a decrease in the response of *Ae. aegypti* to increased extract concentration with increased larval age (i.e., from instar 1 thru instar 4) and/or larval size. In addition, the fitted slope for the combined extract was 30% steeper than the slope for the *A. indica* or *A. aspera* extracts alone and differed between 4th instars and pupae by 20%, whereas the respective slopes for 4th instars and pupae exposed to *A. indica* and *A. aspera* extract alone were parallel (i.e., differed by <3%).

Smoke toxicity assay

There was a significant relationship ($F_{4,20} = 2285$; $P < 0.0001$) between plant extracts and mortality of females exposed to smoke that blood fed on a restrained chick. Sixty-four percent of females blood fed when exposed to smoke from the negative control, whereas 17% blood fed when exposed to smoke from coils containing *A. aspera* extract and to smoke from the positive control (0.2% d-allethrin) (Table 2). The mortality of females exposed to smoke with *A. indica* or *A. indica* + *A. aspera* extract that blood fed was significantly higher than for females exposed to smoke with *A. aspera* extract and to the positive control. Among the 79% of females that failed to blood feed, mortality from exposure to smoke with plant extract averaged 26% and survival averaged 53%. Less than 1% of females exposed to smoke form the negative control died.

Table 1
Mortality of *Aedes aegypti* larvae and pupae to the extracts of *Acalypha indica*, *Achyranthes aspera*, and *Acalypha indica* + *Achyranthes aspera* leaves.

Mosquito life stage	Parameter		LC_{50} (ppm)	
	Slope (±SE)	χ^2	Estimate	(95% Fiducial limit)
Acalypha indica				
$Instar_1$	0.004452 ± 0.000313	2.46	292.5	277.0 to 309.7
$Instar_2$	0.004062 ± 0.000313	3.60	327.9	309.2 to 349.9
$Instar_3$	0.003933 ± 0.000319	4.11	365.3	342.2 to 393.0
$Instar_4$	0.003543 ± 0.000326	2.36	420.6	390.1 to 461.9
Pupa	0.003600 ± 0.003340	2.80	467.6	430.8 to 518.0
Achyranthes aspera				
$Instar_1$	0.004173 ± 0.000308	4.28	283.1	266.9 to 300.7
$Instar_2$	0.004001 ± 0.000311	3.45	322.2	303.7 to 344.1
$Instar_3$	0.003799 ± 0.000314	4.91	355.3	333.4 to 382.6
$Instar_4$	0.003406 ± 0.000319	4.75	409.1	378.9 to 449.6
Pupa	0.003358 ± 0.000331	2.04	456.1	419.1 to 508.1
Acalypha indica + *Achyranthes aspera*				
$Instar_1$	0.007571 ± 0.00057	31.9[a]	207.3	190.3
$Instar_2$	0.006642 ± 0.000341	19.9	234.6	224.0 to 245.2
$Instar_3$	0.006162 ± 0.000333	21.9	253.8	242.6 to 265.2
$Instar_4$	0.005984 ± 0.000333	21.3	277.6	265.9 to 289.8
Pupa	0.004947 ± 0.000453	24.9[a]	326.3	302.1 to 352.9

[a] Significant at $P = 0.05$; heterogeneity factor used in calculation of confidence limits.

Table 2
Effect of exposure to smoke from mosquito coils containing extract (10 g/2.16 × 10^5 cm^3 per 24 h) of *Acalypha indica*, *Achyranthes aspera*, and *Acalypha indica* + *Achyranthes aspera* leaves on blood feeding in *Aedes aegypti*.

Treatment	Percent ± SE[ab]		
	Blood fed	Unfed – alive	Unfed – dead
Acalypha indica	19.4 ± 0.2 a	54.8 ± 0.4 a	25.8 ± 0.5 a
Achyranthes aspera	17.2 ± 0.4 b	53.6 ± 0.2 ab	29.2 ± 0.5 b
Acalypha indica + *Achyranthes aspera*	23.2 ± 0.5 c	51.7 ± 0.7 b	25.1 ± 0.6 a
Positive control[c]	16.8 ± 0.2 b	48.6 ± 0.5 c	34.6 ± 0.4 c
Negative control[d]	64.2 ± 0.6 d	34.9 ± 0.3 d	0.9 ± 0.3 d

[a] 5 replicates per treatment, 50 female mosquitoes per replicate.
[b] Means within each column followed by the same letter are not significantly different (Tukey's HSD test; $P = 0.05$).
[c] Mortein 0.2% d-allethrin 8 Hour Green Coil® (PT Reckitt Benckiser Indonesia, Artha Graha Building, Jl.Jend. Sudirman Kav. 52-53 Jakarta 12190, Indonesia).
[d] Coil made from sawdust and coconut shell charcoal (see text for details).

Field trial

There was a significant relationship between the treatment of water storage tanks with plant extract and a decline in the number of larvae and pupae in the tanks on days 1 thru 5 post-treatment ($F_{5,24} = 81.2$, $P < 0.0001$ for all extracts and life stages) (Table 3). In each test, the mean number of larvae and pupae observed on days 1 thru 5 post-treatment was significantly lower than the numbers of each life stage observed prior to treatment of the water with extract. Decreased mosquito numbers were most apparent in the water tank treated with the *A. indica* + *A. aspera* extract, wherein larval and pupal populations on day 5 post-treatment had declined by 97% and 81%, respectively. During the same time, the numbers of larvae and pupae in the water tank treated with the *A. indica* extract declined by 49% and 58%, respectively, and the numbers in the tank treated with *A aspera* extract declined by 67% and 47%, respectively.

Discussion

Acalypha indica and *A. aspera* are indigenous to Africa and to temperate and tropical Asia (*A. aspera* is also native to Australia, Europe, and South America) and are widely naturalized elsewhere (USDA, 2010). Each species is widespread and locally abundant in its habitat. These facts favor consideration of each plant species for use in mosquito vector control strategies in tropical developing countries where reliance on complex and expensive external inputs is undesirable (Curtis, 2000).

A. indica and *A. aspera* have a documented history of medicinal usage. Decoctions of leaves of the *A. indica* (Tamil lexicon: Kuppaimeni [Ragupathy and Newmaster, 2009]) have been used to treat ear pain, snake bite, scabies infestations, rheumatic arthritis, and syphilitic ulcers (Samy et al., 1999; Ragupathy and Newmaster, 2009). Other aerial plant parts and roots have been used as an expectorant, to treat asthma and pneumonia, and as an emetic, a purgative, and an antihelminthic (Hungeling et al., 2009). Similarly, leaf extract of *A. aspera* (Tamil lexicon: Nagarasi [Ragupathy and Newmaster, 2009]) is fed to newborn babies along with palm jiggery and the inflorescence is scratched against the mother's breasts to increase lactation (Ragupathy and Newmaster, 2009). Pills (1–2 g each) made from

Table 3
Mean number (±SE) of *Aedes aegypti* larvae and pupae per dipper sample in water storage tanks treated with 100 g (200 ppm) of extract of *Acalypha indica*, *Achyranthes aspera*, and *Acalypha indica* + *Achyranthes aspera* leaves.

		Treatment					
		Acalypha indica[a]		*Achyranthes aspera*[b]		*Acalypha indica* + *Achyranthes aspera*[c]	
Sampling interval		Larvae[d]	Pupae	Larvae	Pupae	Larvae	Pupae
Pretreatment	Day 0	80.2 ± 3.4 a	69.2 ± 7.8 a	70.4 ± 1.7 a	64.2 ± 4.0 a	53.4 ± 1.9 a	31.8 ± 1.4 a
Posttreatment	Day 1	59.6 ± 3.4 bc	37.4 ± 3.2 b	39.4 ± 2.1 b	39.8 ± 1.2 b	7.8 ± 0.6 b	8.8 ± 1.2 b
Posttreatment	Day 2	62.6 ± 2.1 b	38.8 ± 2.3 b	35.4 ± 1.9 bc	41.0 ± 1.9 b	6 ± 0.4 bc	7.4 ± 1.2 b
Posttreatment	Day 3	51.8 ± 1.9 c	29.6 ± 2.1 b	33.6 ± 3.1 bc	41.4 ± 1.6 b	5.2 ± 0.7 bc	7.2 ± 0.9 b
Posttreatment	Day 4	52.0 ± 2.4 c	27.4 ± 1.2 b	29.2 ± 1.6 cd	38.6 ± 2.2 b	3.0 ± 0.7 c	4.4 ± 0.9 b
Posttreatment	Day 5	40.6 ± 1.5 d	28.4 ± 2.2 b	23.2 ± 2.1 d	33.6 ± 1.8 b	1.2 ± 0.7 d	5.8 ± 1.5 b

[a] Vadavalli (Village), Tamil Nadu, India; water (≈0.5 m^3) storage tank, 2 m long × 1 m wide × 2 m deep.
[b] Kalveerampalayam (Village), Tamil Nadu, India; water (≈0.5 m^3) storage tank, 1 m long × 1 m wide × 2 m deep.
[c] Ponmanapalayam (Village), Tamil Nadu, India; water (≈0.5 m^3) storage tank 1 m long × 1 m wide × 1 m deep.
[d] Tabulated means based on raw data, each observed datum transformed to log10 (n + 1) prior to statistical analysis. Posttreatment means within each column followed by the same letter as the pretreatment mean are not significantly lower than the pretreatment mean (Dunnett's one-sided t test, P = 0.05).

crushed leaves of *A. aspera* are applied to boils until healed (Sajem and Gosai, 2006) and leaf extracts of this species are used to treat dropsy (Samy et al., 1999).

In the laboratory, ethyl acetate extracts of *A. aspera* leaves tested against 4th instar *Anopheles subpictus* Grassi and *Culex tritaeniorhynchus* Giles resulted in LC_{50} values of 49 ppm and 68 ppm, respectively (Zahir et al., 2009). Bagavan et al. (2008) used bioassay-guided fractionation of *A. aspera* leaf extract to isolate a saponin with an LC_{50} against early 4th instar *Ae. aegypti* and *Culex quinquefasciatus* Say larvae of 18 ppm and 27 ppm, respectively.

Acalypha indica has been studied as a botanical insecticide against mosquitoes less than *A. aspera*. When tested against *An. stephensi* Liston, the methanol extract of *A. indica* leaves produced ovicidal activity 120 h after treatment and an LC_{50} of 15 ppm against 4th instars (Govindarajan et al., 2008). Crude extracts of *A. indica* tested at 1000 ppm against early 4th instar *Ae. aegypti* resulted in 15% (solvent: ethyl acetate) to 66% (solvent: hexane) mortality after 24 h, depending on the solvent used (acetone [52%], chloroform [25%], methanol [48%]) for extraction (Bagavan et al., 2008), although dosimetry studies were not made.

In other studies of the toxicity of phytochemicals to larval *Ae. aegypti*, the LC_{50} for ethanol root bark extracts of *Annona crassiflora* Mart. (Annonaceae) is <1 ppm (Omena et al., 2007); 9 ppm for petroleum ether extracts of the leaves of *Jatropha curcas* L. (Euphorbiaceae); 81 ppm for methanol extracts of *Ficus benghalensis* L. (Moraceae) leaves (Govindarajan, 2010); and 426 ppm and 554 ppm, respectively, for the acetone extracts of the leaves of *Ocimum sanctum* L. (Lamiaceae) (Anees, 2008) and *Trichosanthes anguina* L. (Cucurbitaceae) (Rahuman et al., 2008). This wide range of toxicities can be attributed to differences in the taxonomic status of the plants used for analysis and to the life stage and vigor of the mosquitoes used for testing. Biological activity of phytochemicals also varies with the plant part (i.e., leaves, stems, bark, roots, flowers, fruit), the solvents used for extraction, the geographic location from which the plant specimens are gathered, and the methods and conditions applied to plant nutrition and culture (Sukumar et al., 1991).

The most effective larvicide in our study was the combined extract of *A. indica* and *A. aspera*. The LC_{50} for 1st and 4th instars was 207 ppm and 277 ppm, respectively. Both of these were 30% lower than for the *A. indica* and *A. aspera* extracts alone. The combined extract was also the most effective pupicide, with an LC_{50} (326 ppm) 30% lower than for either extract alone.

As expected, smoke from the coil with 0.2% d-allethrin killed significantly (20%) more *Ae. aegypti* than smoke from the plant extract coils. There was no synergistic effect on mortality from exposure to *A. indica* + *A. aspera* extract smoke as noted by Murugan et al. (2003) when exposing *Ae. aegypt* to smoke from coils with *O. basilicum* + *A. amara* extract. Smoke from burning coils that contained *A. aspera* extract were as effective as those with 0.2% d-allethrin for preventing blood feeding by *Ae. aegypti*. This finding is remarkable from a practical standpoint (despite the increased amount [275×] of *A. aspera* extract required for such efficacy), given the availability and low cost associated with collection of *A. aspera* in many areas of the world and the relative ease with which the extract can be incorporated into mosquito coils. Additional refinement of *A. aspera* extract as a component of mosquito coils (using the approach noted above for larvicide development) may also result in improved smoke toxicity effects (increased inhibition of blood feeding and mortality) in female *Ae. aegypti*.

Our purpose in making field tests of the plant extracts was to determine their effectiveness as mosquito larvicides and to assess the practicality of using native plant materials to kill mosquitoes. From a technical standpoint, the reduction in mosquito numbers we attribute to plant extract effects in these tests is confounded with the change in mosquito numbers in untreated water containers as we did not estimate the latter parameter. Nevertheless, the decrease in mosquito larvae observed in all water tanks on day 1 post-treatment (*A. indica*: 25%; *A. aspera*: 44%; *A. indica* + *A. aspera*: 85%) is strongly suggestive of plant extract effects, as is the relatively greater rate of decrease in mosquito numbers in the water storage tank treated with combined extract by day 5 post-treatment (97%) compared with the decrease of larval numbers in tanks treated with the *A. indica* (49%) or *A. aspera* extract (67%) only during the same time period.

The results of our study indicate that further evaluation of the combined *A. indica* + *A. aspera* extract as a mosquito larvicide is warranted. Mosquito coils with *A. aspera* extract also show promise as a practical and economical means for mitigating mosquito blood feeding. Given the eventuality of further development of these phytochemicals, particular attention should be given to plant culture and nutrition conditions, the use of bioassay-guided fractionation to isolate and identify the most active phytochemical constituents, and, in the case of mosquito larvae, the potential for combining botanical, synthetic and/or microbial larvicides for mosquito control (Murugan, et al., 2003; Harve and Kamath, 2004).

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Ethical approval

The use of animals in this study was approved by the Indian Council of Medical Research, New Delhi, India; registration number 722/02/b/BTCS EA.

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