

# Toxicity of *Spathodea campanulata* P Beauvois (Scrophulariales: Bignoniaceae) aqueous extracts against immature stages of *Anopheles albimanus* (Diptera: Culicidae) under laboratory conditions

Jose Luis Torres-Estrada<sup>1</sup>  
 Julio Cesar Velazquez Gonzalez<sup>1</sup>  
 Silvany M Rios Delgado<sup>1</sup>  
 María Guadalupe Vázquez-Martínez<sup>1</sup>  
 R Patricia Penilla-Navarro<sup>1</sup>  
 Americo D Rodriguez<sup>1</sup>

<sup>1</sup>Centro Regional de Investigación en Salud Pública, Instituto Nacional de Salud Pública, Colonia Centro, Tapachula, Chiapas, México

**Purpose:** To determine the effects of African tulip *Spathodea campanulata* aqueous extracts on every immature stage of *Anopheles albimanus* under laboratory conditions.

**Methods:** The extract was obtained making an incision on the apical part of prefloral bulbs, and two sets of dilutions with distilled water were prepared. The first set was used at 50%, 20%, 10%, 5%, and 2.5% concentrations in bioassays to test its effect on egg-hatching inhibition. The second set was used at 10%, 5%, 1%, 0.1%, and 0.01% to test toxicity on larvae and pupae. Also, residual efficacy and lethal time (LT) were estimated.

**Results:** The highest inhibition (87.5%) recorded for egg hatching was at a 50% concentration. Third and fourth instar larvae and pupae were the most susceptible to 10% and 5% of *S. campanulata* aqueous extracts, with 98.3%–100% mortality. The residual activity with 10% concentration persisted 7 days, with 100% mortality, and LT for 99% mortality (LT<sub>99</sub>) was 2.28 hours on third instar larvae, 1.7 hours on fourth instar larvae, and 2.25 hours on pupae.

**Conclusion:** *S. campanulata* extracts are promising as biolarvicides. Further toxicological and chromatographic studies are encouraged and needed.

**Keywords:** African tulip, botanical insecticides, malaria, mosquitoes

## Introduction

Malaria is still a major vector-borne disease affecting humans. *Anopheles albimanus* Wiedemann is the main malaria vector in coastal areas in Mexico and Central America.<sup>1</sup> The use of insecticides continues to be the most effective control method; however, this species has developed multiple resistance to most insecticides used by public health institutions, which can hinder the control of malaria in endemic areas.<sup>2–5</sup> The use of insecticides can also damage the environment and a wide variety of nontarget organisms.<sup>6</sup> Based on such difficulties and limitations in the use of insecticides for the control of mosquito vectors, the search for new and less aggressive alternatives, such as toxic compounds from plant extracts, is a good choice.<sup>7,8</sup>

Several plant extracts that have served as a basis in developing synthetic insecticides have been tested in the past. *Spathodea campanulata* (African tulip tree) could be a good candidate for *A. albimanus* control, since it has been proven to contain an alkaloid toxic to *Aedes fluviatilis* larvae.<sup>9,10</sup> However, the effectiveness against the immature stages of the malaria mosquito has not been tested. *S. campanulata* is an established introduced species currently widely distributed in Mexico as an ornamental tree.

Correspondence: María Guadalupe Vázquez-Martínez  
 Centro Regional de Investigación en Salud Pública, Instituto Nacional de Salud Pública, 4a. Norte y 19 Poniente s/n, Colonia Centro, C.P. 30700, Tapachula, Chiapas, México  
 Tel + 52 962 6250044  
 Fax +52 962 6265782  
 Email mguadalu@insp.mx

Herein, we present the results about the efficacy of *S. campanulata* aqueous extracts against immature stages of *A. albimanus*.

## Materials and methods

### Mosquitoes

Immature stages (eggs, first, second, third, and fourth instar larvae and pupae) were obtained from a colony established in the Centro Regional de Investigacion en Salud Publica and maintained at  $28 \pm 2^\circ\text{C}$  temperatures and  $75 \pm 5\%$  relative humidity.<sup>11</sup>

### Aqueous extracts

Prefloral bulbs of *S. campanulata* were collected from cultivated trees in the area of Tapachula City, Chiapas, Mexico. The extract was obtained by making an incision on the apical part of the bulbs, and the liquid obtained was kept in 1 L glass containers. Two sets of dilutions with distilled water were prepared, one to test whether an effect on egg-hatching inhibition exists, and the second to test toxicity on larvae and pupae.

### Egg-hatching inhibition

The efficacy of 50%, 20%, 10%, 5%, and 2.5% concentrations of *S. campanulata* extracts to inhibit *A. albimanus* eggs from hatching was examined. For each concentration, four replicates of 20 eggs were exposed to *S. campanulata* in plastic containers. As a control, five replicates (one for each concentration) of 20 eggs were exposed to distilled water without *S. campanulata* extracts. Egg hatching in containers was recorded at 24 and 48 hours, using a dissection microscope to observe the first instar larvae.

### Toxicity against larvae and pupae

Bioassays to determine the lethal concentration of the *S. campanulata* extracts (at 10%, 5%, 1%, 0.1%, and 0.01%) were conducted following the World Health Organization protocol to test susceptibility on mosquito larvae (first, second, third, and fourth instar) and pupae.<sup>12</sup> In total, 6400 larvae were used for the bioassays: four replicates of 20 larvae per container (80) per each instar (320) per each one of the five concentrations (1600), with four experiment replicates (6400 in total). For the pupae bioassays, one experiment was carried out with the five concentrations mentioned above with four replicates, using 400 pupae. As a control, for each experiment, larvae and pupae were exposed to distilled water without the *S. campanulata* aqueous extract. Mortality was assessed 24 hours after the treatment.

## Residual efficacy of aqueous extracts

To determine the residual efficacy of *S. campanulata* aqueous extracts, 100 of the most susceptible instar larvae were exposed every day to the concentration with the highest mortality, and distilled water without the aqueous extract was used as a control. Mortality was recorded daily, and dead individuals were replaced in the treatment until the observed mortality decreased to less than 80%. A new set of individuals were replaced every day in the control containers according to the treatment. This experiment was carried out in two replicates.

### Lethal time

The lethal time for 99% mortality ( $LT_{99}$ ) was determined at 0.5, 1.0, 1.5, 2.0, and 24 hours in 250 mL of the *S. campanulata* aqueous extract, with the highest mortality reported in each immature stage.

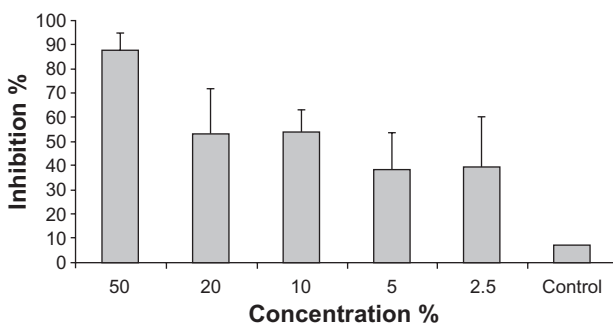
### Statistical analysis

The percentage of larval mortality and egg-hatching inhibition was calculated, and the data were normalized by transformation to the arcsine square root.<sup>13</sup> The efficacy of each concentration was analyzed by a two-way analysis of variance (ANOVA) and Tukey's multiple contrasts tests.<sup>14</sup> LT results were analyzed with a Probit test (version 1.5).

## Results

### Egg-hatching inhibition

All the *S. campanulata* aqueous extracts tested inhibited egg hatching (36%–87.5%) (see Figure 1), with significant differences observed against the control ( $P < 0.05$ ). Post hoc analysis revealed that exposure to the highest concentration (50%) resulted in statistically higher inhibition ( $87.5 \pm 7.1\%$ ) compared with the rest of the concentrations ( $F = 40.39$ ;  $df = 8$ ;  $P = 0.0001$ ).



**Figure 1** Egg-hatching inhibition by *Anopheles albimanus* caused by several concentrations of *Spathodea campanulata* aqueous extracts.

## Toxicity against larvae and pupae

Toxicity of *S. campanulata* aqueous extracts statistically varied according to the concentration tested and the immature stage of *A. albimanus* ( $P < 0.05$ ) (see Figure 2). Although the lowest mortality was observed always in the first instar stage compared with the rest of the immature stages in any of the extract concentrations tested ( $P < 0.05$ ), its highest mortality ( $36.6 \pm 15.8\%$ ) was also recorded with the highest concentration aqueous extract tested (10%) (see Figure 2). No statistical differences in percentage of toxicity were observed between the 10% and the 5% concentrations, but differences were observed between these concentrations and the rest of the concentrations (1%, 0.1%, and 0.01%;  $P < 0.05$ ) (see Figure 2). Similar percentage mortalities were found with the 10% and the 5% extract concentrations ( $P > 0.05$ ) for the second (91.6% and 89.9%, respectively), third (98.43% for both), fourth (100% and 97.5%, respectively), and pupae (100% for both) stages.

## Residual efficacy of aqueous extracts

The fourth instar stage was the most susceptible at the 10% concentration. The *S. campanulata* 10% aqueous extract caused 100% mortality in this *A. albimanus* instar during 7 days, decreasing to 80% after the eighth day (Figure 3).

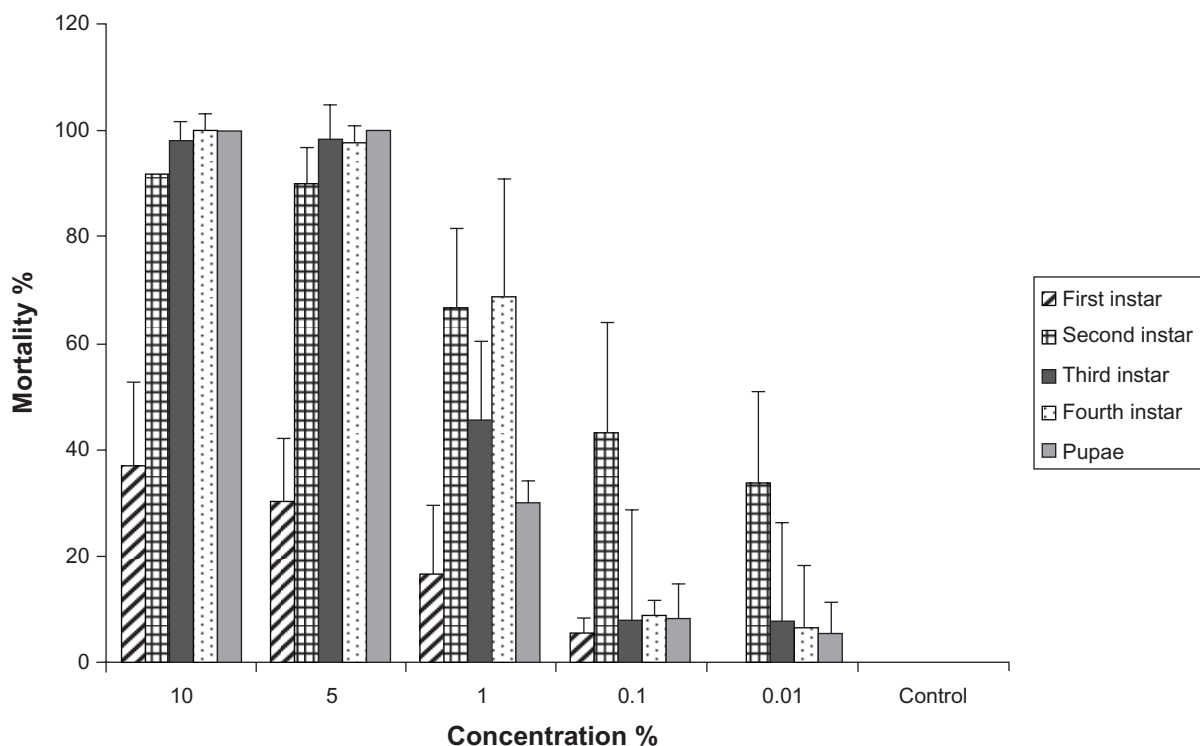
## Lethal time

The  $LT_{99}$  of 10% *S. campanulata* aqueous extracts determined was 2.28 hours, when 99% of the third instar larvae population died after exposure. This extract concentration also killed 99% of the fourth instar larvae and pupae population after 1.7 hours and 2.25 hours, respectively; while the first and second instar larvae population required 63.8 and 25.9 hours, respectively (Figure 4).

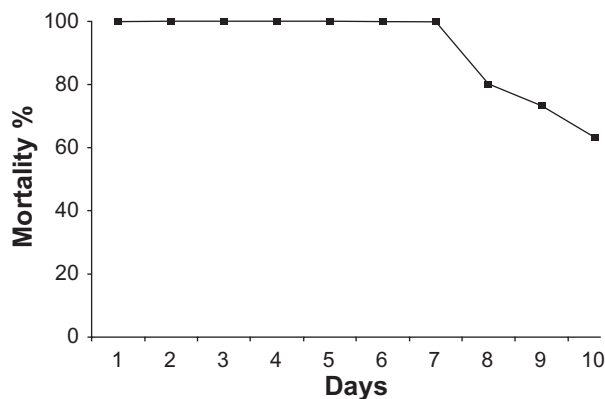
## Discussion

Aqueous extracts were tested on *A. albimanus* egg, larvae, and pupae; the eggs being the less susceptible. The aqueous extracts at 10% and 5% concentrations killed between 89.9% and 100% of the *A. albimanus* immature stage populations, except for the first instar larvae stage, where mortality ranges were less than 40%. However, we highlight the effect with both extract concentrations on the pupae stage, where 100% mortality was obtained for all replicates.

Toxicity of African tulip flowers has been reported for Meliponinae, Diptera, Vespidae, Formicidae, and Orthoptera.<sup>15</sup> Particularly, the effects of *S. campanulata* on *A. fluviatilis* were previously reported, as its antimalarial activity with stem bark extracts was also reported on *Plasmodium berghei berghei* in mice.<sup>9,16</sup> Our results have demonstrated that *S. campanulata* is a promising product



**Figure 2** Effects of *Spathodea campanulata* aqueous extracts on immature stages of *Anopheles albimanus*.

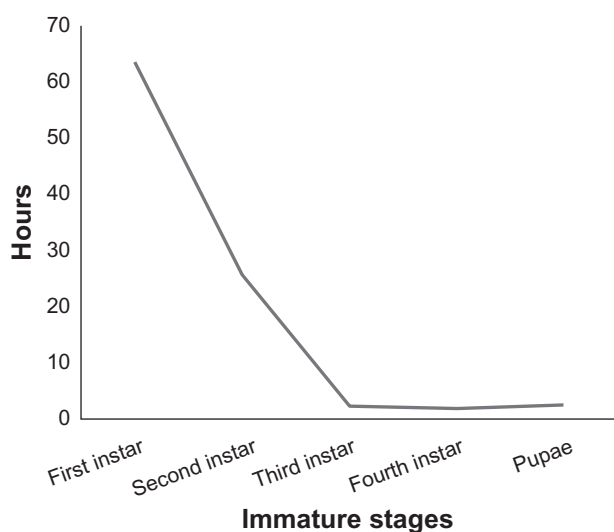


**Figure 3** Residual efficacy of *Spathodea campanulata* aqueous extracts on fourth instar larvae exposed at a 10% concentration.

for malaria vector control in Mexico and elsewhere, where *A. albimanus* may still be causing problems.

Egg-hatching inhibition was previously reported on several mosquito species caused by terpenic compound extracts from various plants.<sup>17</sup> Also, an ovicide effect of *Azadirachta indica*, a plant with various properties such as antifeedancy, growth regulation, fecundity suppression, male sterility, oviposition repellency, changes in biological fitness such as loss of flying ability, immunodepression, enzyme inhibition, splitting of biological rhythms, and so forth against insects has been reported against *Culex quinquefasciatus* and *C. tarsalis*.<sup>18,19</sup> In this study, although at a high concentration (50%), aqueous extract of *S. campanulata* caused 87.5% egg-hatching inhibition.

LT observed on the different immature stages of *A. albimanus* were consistent with our susceptibility



**Figure 4** Lethal time of *Spathodea campanulata* aqueous extracts on different instar larvae and pupae exposed at a 10% concentration.

results, where the latest stages (third and fourth instar and pupae) were more susceptible to the 10% concentration, giving the  $LT_{99}$  at 2.28, 1.7, and 2.25 hours, respectively, as compared with first and second instar, which needed 63.8 and 25.9 hours, respectively. It is clear that the potency of the extract is higher at later instars, which suggests that at the earlier stages the presence of a specific age-related protein may give protection against the active compound. Further proteomic studies could help to elucidate the level at which the extract is acting.

Larvicidal activity of *Quillaja saponaria* commercial bark saponin extract, which was studied on third and fourth instar larvae of *A. aegypti* and *C. pipiens*, showed 100% mortality after 1–5 days at 800 and 1000 mg/L doses, respectively.<sup>20</sup> Other studies under laboratory and semi-field conditions<sup>21</sup> demonstrated that the activity of the *Calophyllum inophyllum* seed and leaf extracts as the leaf extracts of *R. nasutus* persisted for up to 10 days. On the other hand, insect growth regulator novaluron-controlled immature *Culex* mosquitoes for up to 14 days at 1.25, 2.5, and 5 ppb in the microcosms, and for up to 7 days using 1, 5, and 10 mg/m<sup>2</sup> doses.<sup>22</sup> The larvicide effect of 10% concentration of *S. campanulata* extract on fourth instar *A. albimanus* lasted 7 days, which is in between the residual efficacy of the novaluron and the *Q. saponaria* extract. Further studies are needed to purify the extract and get a more concentrated active compound as well as to discover the active ingredient or ingredients for its synthesis. Either approach would lead to the possibility of formulating a more operationally feasible product.

## Conclusion

The results of this study suggest that *S. campanulata* aqueous extracts are promising as larvicides against *A. albimanus* and might be useful in the search for new natural larvicidal compounds. However, more studies are needed regarding the mode of action and identification of the active compounds in *S. campanulata*, as well as its effects on nontarget organisms. Once its impact on nontarget organisms is elucidated, and given the relatively easy way to prepare *S. campanulata* aqueous extracts, a community engaged approach could be implemented as an alternative in villages within endemic malaria areas of Mexico and worldwide. This would help to empower villagers and could be included as an innovative component of control programs where community involvement and reduction of chemical control are encouraged.

## Acknowledgment/disclosure

This study was supported by Centro Regional de Investigación en Salud Pública/Instituto Nacional de Salud Pública. The authors report no conflicts of interest in this work.

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