Colour Cues: Effects of *Ipomoea* Plant Extract on *Culex quinquefasciatus* Say Gravid Females in Choosing Oviposition Site

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**Abstract:** The interaction between plant and insect is dynamic which may favor either the plant or insect. Plant chemicals deeply implicated in this relationship and influence the insect behavior. Here, we investigated oviposition behavior response of *Culex quinquefasciatus* mosquitoes based on the colour cues due the colour produced by *Ipomoea cairica* leaves extract. In this study, two set of oviposition choice experiments were conducted; (1) single solution in a cage, (2) multiple concentration solutions in a cage. In single solution experiment, only one available oviposition site was offered to five gravid females and in multiple concentration tests, four available oviposition sites were offered to twenty gravid females. The tested concentrations were set up at 100ml of (1) control (distilled water only), (2) 50ppm, (3) 150ppm, and (4) 300ppm of *I. cairica* plant extracts. The highest concentration of 300ppm appeared to show highest intensity with darkest color followed by 150ppm and 50ppm concentrations. More gravid females were found drowned in the highest concentration, 300ppm of acethonilic leaves extract compared to 150ppm and 50ppm of the tested extract. No eggs were found in all tested solutions. The studied extract was found to effectively attract gravid *Cx. quinquefasciatus* females and subsequently cause mortality and inhibiting egg deposition. The interference caused by the acethonilic extract of *I. cairica* on the oviposition activity of *Cx. quinquefasciatus* can result in better control of the insect vector.
**INTRODUCTION**

There has been an increased interest in the manipulation of pest behaviour in order to achieve better pest management and reduce dependency on broad spectrum insecticides (Foster and Harris 1997). Using plant resources for deliberate manipulation of pest behaviour can be applied in pest management programs. Plants have coevolved with insects and have produced secondary compound to deter phytophagous insects. Secondary compound from plants have been identified to be toxic to arthropod pests. Although the primary function of these compounds is for defense against phytophagous insects, many of them are also effective against mosquitoes and other biting Dipteran (Pichersky and Gershenzon 2002) as repellent, larvicide or adulticide.

Phytochemicals from plants are important for mosquito oviposition attractants and stimulants. However, they can also have a negative or deterrent effect on oviposition (Bentley and Day 1989). According to Waliwitiya et al. (2009), the phytochemical tested; thymol, pulegone, citronellal and eugenol showed strong repellent/deterrent activity, whereas β-pinene, borneol acetate, borneol and camphor acted as strong oviposition attractants. The leaf extract of *Solanum trilobatum* at 0.1 % concentration greatly reduced the number of eggs deposited by gravid *An. stephensi* by 90–99% (Rajkumar and Jebanesan 2005). The essential oil of *Cinnamomum zeylanicum* was found to have oviposition-deterrent potential against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Prajapati et al. 2005). Rajkumar and Jebanesan (2009) have reported that the oviposition deterrence effects of ethanolic leaf extract of *Cassia obtusifolia*.

Various other cues are also involved in mediating a wide range of mosquito behaviors which includes the recognition of specific features of the oviposition sites (Mboera et al. 2000). The location and selection of an oviposition site involves visual, olfactory, and tactile responses. The ovipositional flight is triggered by environmental factors, especially rainfall, relative humidity, temperature, and wind speed (Bentley and Day 1989). Oviposition sites selection by gravid female
mosquito is very important to determine the resources available for the aquatic immature stages to develop into adulthood. The selection of appropriate oviposition site is particularly important in mosquito species, since the juveniles are unable to move to a better habitat if the condition becomes unfavorable (Spencer et al. 2002). It is essential throughout ontogeny of all mosquito species for continuous offspring survival in specific location.

Multiple researches had been conducted concerning mosquito ovipositional behavior caused by different possible stimuli. Based on a study done by Chadee et al. (1990), it was reported that Ae. aegypti mosquitoes avoided sites containing eggs laid by themselves or by conspecifics. Bacteria present in the organic-rich water were found to produce chemicals that are highly attractive to gravid mosquitoes (Reiter et al. 1991; Copeland and Craig 1992; Allan and Kline 1995). The presence of predatory tadpole shrimp (Triops) deterred oviposition by the mosquito Cx. quinquefasciatus due to the physical disturbance created by predator movement (Tietze and Mulla 1991). The chemicals released from discarded cigarette butt microcosms tended to cause more egg deposition by Ae. albopictus females as decomposition progressed but somehow kill newly hatched larvae in the oviposition site (Dieng et al. 2011). Gravid Cx. quinquefasciatus females were found to locate suitable oviposition sites by odour orientation associated with high organic content and lay their eggs in clusters, called egg rafts, on the water surface (McCall and Eaton 2001). Their preference for moderately aerated, polluted water which is rich in decaying organic matter for oviposition sites have made Cx. quinquefasciatus a dangerous vector because these sites are usually prevalent near human habitation (Blackwell et al. 1993).

Ipomoea L. from family Convolulaceae has been chosen in this study which comprises of about 650 species and it is distributed all over the world (Mabberley 2008; Austin and Huan’man 1996). Ipomoea (commonly known as ‘morning glory’) is a cosmopolitan climbing genus from warm and pan tropical regions. Its members have large showy flower in trumpet shaped with different colours mainly white, purple, blue, pink, and red (Cronquist 1981). Ipomoea cairica is commonly known as ‘Railway creeper’, and being ornamental in nature it is widely used in fencing in domestic and peri-domestic situations (Thomas et al. 2004). Ipomoea cairica reproduces easily through both sexual and asexual propagation (Liu et al. 2006). This plant has been known for the medicinal
properties, in which a drink made from the plant's crushed leaves is used for the treatment of body rashes, especially those accompanied by fever (Watt and Brandwijik 1962). Topical repellency effects of the essential oil extracted by steam distillation from leaves of I. cairica were evaluated and found to be highly toxic to the larvae of Cx. tritaeniorhynchus followed by Ae. aegypti, An.stephensi, and Cx. quinquefasciatus (Thomas et al., 2004; Rajkumar and Jebanesan 2007).

In the current study, we tested the effects of acethoniilic extract of Ipomoea cairica leaves on the oviposition behavior response of vector urban bancroftian filariasis, Cx. quinquefasciatus gravid female mosquitoes. We hypothesized that the highly concentrated plant extract solution will attract more Cx. quinquefasciatus gravid female to oviposit their eggs and also cause mortality to the gravid females. We also examined the interactive component of reception visual stimuli caused by I. cairica plant that may become an attractant to Cx. quinquefasciatus gravid females in choosing oviposition site.

MATERIALS AND METHODS

Mosquito colonies - Wild strain of Cx. quinquefasciatus larvae were collected from drain containing stagnant water in Bagan Dalam area, Penang, Malaysia (5° 24' 00"N, 100° 23'00"E). Collected larvae were reared in enamel trays containing decolorinated tap water and fed with fine powder mixture of dog biscuit, beef liver, yeast and milk powder at ratio of 2:1:1:1 by weight. The pupae were transferred into plastic containers and placed in screened cages (23×23×32cm) to allow emergence of adults. Adult mosquitoes were continuously provided with 10% sucrose solution in a vial with a cotton wick. On the day 5 after adult emergence, females were allowed to feed overnight on a restrained laboratory mouse. Three days after blood feeding, gravid females from these colonies were used as subject in oviposition assay experiments. The mosquito colonies were maintained under laboratory condition at a temperature of (28±2° C) and (80±10%) relative humidity (RH).
Preparation of plant extracts - Samples of *I. cairica* leaves were collected from residential area in Relau, Penang, Malaysia (5°25′00″N 100°19′00″E) and were identified by Botanical Department of Universiti Sains Malaysia. Crude extract was made by grinding the air dried leaves using electrical stainless steel blender and extracted with acetone using Soxhlet apparatus. Extracts were then removed the excess solvent using rotary vacuum evaporator. The solvent from the concentrated crude extract were further removed by placing it in electrical oven at 37°C for a week. Stock solution was prepared by dissolving one gram of crude extract in 100 ml of solvent and subsequently tested concentration of 50ppm, 150ppm and 300ppm were prepared using serial dilutions technique.

Colour cues experiment - To investigate the colour cues from plant extract, we used spectrographic analysis. We prepared different concentrations of the tested plant extract solution comprising of 50, 150 and 300ppm and compared with control as reference (distilled water) in order to test the reflectance properties of *I. cairica* color solutions. By using a USB Ocean Optics 2000 spectroradiometer and Xenon Pulse X2 lamp (Ocean optic) as light source, we compared the plant color solution based on the reflectance and whether this affected the choice of oviposition site by *Cx. quinquefasciatus* gravid females. The fiber optics probe was mounted inside amatte black plastic tube to exclude ambient light. The distance between each object and the probe was fixed at 1 cm. While, the angle of illumination and reflection was fixed at 45° to minimize glare. Spectra were calculated at 5 nm intervals from 300 to 700 nm with SpectraSuite software.

Oviposition experiments - In this study, two different set of oviposition choice experiments were conducted; (1) Single solution in a cage, (2) Multiple concentration solutions in one cage. Single and multiple concentrations of acetone extract of *I. cairica* leaves were assessed for their effects on selection of oviposition site of *Cx. quinquefasciatus* gravid females. Both experiments were designed in order to understand the possible effects caused by the acethonilic extract of *I. cairica* leaves on oviposition site selection by gravid *Cx. quinquefasciatus* females. Tests were carried out under laboratory conditions at 28±2 °C and 80±10 % relative humidity (RH).

In single concentration solution experiment, five gravid females were transferred to each screened mosquito cages (23×23×32cm). Each of the cages was offered with only one available
oviposition site and ran separately. Ceramic bowls were used as oviposition site. Each oviposition site consisted of 100ml of different concentrations; (1) control (distilled water without any of plant extract solution), (2) 50ppm, (3) 150ppm, and (4) 300ppm of *I. cairica* plant extract. Three replicates for each concentration were conducted simultaneously. The cages contained different test concentrations were placed side by side at 0.5m between each other. Data of drowned gravid female were recorded.

In multiple concentration solutions test, twenty gravid females were transferred into a screened cage and four ceramic bowls with 100ml of tested plant extract of (1) control (distilled water only), (2) 50ppm (3) 150ppm and (4) 300ppm of *I. cairica* plant extracts were placed in a same cage. Bowls were positioned in a square shape and position of the bowls was alternated between the different replicates to nullify any bias for site preferences. The experiment was replicated three times. The analysis for measuring the oviposition response in this experiment was based on the number of drowned females rather than egg rafts. This is because no eggs rafts were laid by *Cx. quinquefasciatus* mosquitoes in all treatments.

*Statistical analysis* - Analysis of variance (ANOVA) was conducted separately using SPSS program version 20.0 for the number of drowned females in single and multiple concentration tests to determine significant differences of the extract solution concentration. Data were tested for normality using Kolomogorov-Smirnov test prior to analysis. Results with P<0.05 were considered to have statistically significant. The effects of visual stimuli by *Cx. quinquefasciatus* in choosing oviposition site were represented by the mean number of gravid *Cx. quinquefasciatus* drowned in the given oviposition site.

**RESULTS**

Spectrographic results showed that the acethonilic extracts of *I. cairica* leaves at different concentration have reflectance intensity peaking around 500-600nm. The spectral curve corresponds to the colour of the extract, which is yellowish green. Highest intensity counts were
given by the tested extract at 300ppm concentration followed by 150ppm and 50ppm concentrations of the extract (Fig. 1). The reflectance curves given by the plant extract at the tested concentrations clearly showed difference in color intensity level of the plant extract. The highest concentration, 300ppm of the studied plant extract appeared to show highest intensity with darker colour followed by 150ppm and 50ppm concentrations.

High numbers of gravid *Cx. quinquefasciatus* females were found in oviposition site with highly concentrated and darker color plant extract solution, for both single and multiple concentration solutions tests (Fig. 2). In multiple concentration solutions tests, gravid female were shown to be attracted to oviposit in the plant extract solution, causing death to the females. Similar conditions occurred even when only one solution was made available. No eggs were found in tested solutions except in control.

Greater numbers of gravid females were found significantly drowned in oviposition site with the highest concentration; 300ppm of the tested extract for single solution (df=3, F=12.571, P <0.05; Table 1) and multiple solutions (df=3, F=88.242, P <0.05; Table 1) compared to the lower concentrations. Drowned gravid females were even found at the lowest concentration but at a reduced proportion. No drowned gravid females were observed in control (Table 1). The acethonilic extract of *I. cairica* leaves were found to inhibit egg deposition as no egg rafts were found laid in the treated oviposition site.

**DISCUSSION**

Our study revealed that gravid *Cx. quinquefasciatus* females were attracted to oviposition site with high intensity and darker color of *I. cairica* plant extract compared to control (distilled water). However, no eggs were found in any of the plant extract solutions. Interestingly, more *Cx. quinquefasciatus* gravid females were found drowned and died in the higher concentration of 300ppm of *I. cairica* plant extract. The 300 ppm concentration has the darkest colour and the highest intensity among all treatments. The colour of the water or water clarity is an important factor
influencing oviposition behavior of female mosquitoes (Li et al. 2009). The reflection colour (Barbosa et al. 2007) caused by the container can also attract mosquitoes to oviposit. Li et al. (2009) found that *Culex pipiens pallens* females laid significantly more eggs in dark blue water (50 mg/L of methylene blue solution, or 50 mg/L and 200 mg/L of brilliant blue solution) than in distilled water. *Anopheles quadramaculatus* also oviposited preferentially in oviposition waters that appeared darker than controls in a laboratory experiment (Lund 1942). Black and red were the most preferred colors for oviposition of *Cx. annulirostris* and *Cx. molestus*, respectively. Whereas, yellow and green were the least preferred colors for both species under laboratory condition (Dhileepan 1997). Oviposition waters dyed with ink were detected to be significantly attractive to ovipositing *Cx. quinquefasciatus* when compared to distilled water controls in a laboratory evaluation. The respective study also suggested that dark colors attract more gravid *Cx. quinquefasciatus* because females respond to the increased optical density of the dyed water rather than to the volatile components of the dye (Beehler et al. 1993).

The reflected color of yellowish green at 500-600nm of the acethonilic extract of *I. cairica* leaves may have been detected effectively by *Cx. quinquefasciatus* gravid females. Based on previous study, electroretinographs revealed spectral sensitivity of the eye of female *Ae. aegypti* ranging from ultraviolet (323 nm) to orange-red (621 nm), with sensitivity peaks in the ultraviolet (323–345 nm) and green (523 nm) wavelengths. It was also reported that the eye of female *Ae. aegypti* had relatively poor acuity and capable of some wavelength discrimination but high overall sensitivity to light (Muir et al. 1992). Since *Ae. aegypti* and *Cx. quinquefasciatus* are from the same genus (Culicinae) there could be a similarity of the eye sensitivity peak. We proposed that the color yellowish green of the tested extract can be effectively detected by *Cx. quinquefasciatus* gravid females. We however acknowledged that further comparative study needs to be conducted, for example by comparing the extract concentrations against dyed concentration. We believe that our preliminary result is sufficient to suggest such colour specific preference for oviposition by *Cx. quinquefasciatus*.

Additionally, the organic content of the plant extract solution might also have attracted *Cx. quinquefasciatus* gravid females to oviposit. Previous studies have reported high organic content in
surveillance traps attracted more gravid female mosquitoes. Ovitraps baited with grass infusions, *Panicum maximum* (Jacq.) evaluated in the field were reported to collect significantly more *Aedes* eggs than controls containing tap water (Santana et al. 2006). Bermuda grass (*Cynodon dactylon*), oak leaf (*Quercus virginiana*), acacia leaf (*Acacia schaffneri*) and algae (*Spirogyra* sp.) infusions were found effective in collecting *Cx quinquefasciatus, Cx. nigripalpus*, and *Cx. erraticus* (McPhatter and Debboun 2009). Whereas, bioassays with *Spirogyra majuscula* organic extracts indicated that these extracts attracted more *Anopheles pseudopunctipennis* gravid females under laboratory conditions (Torres-Estrada et al. 2007).

Even though acethonilic extract of *I. cairica* leaves attracted more gravid females, no eggs rafts were laid in the treated oviposition site. The tested plant extract solution caused mortality of the gravid *Cx. quinquefasciatus* females used in our study thus, inhibiting the oviposition activity. The different concentration of studied plant extract may have reduced the water surface tension to a certain extent which prevented proper landing and egg deposition by gravid females. Similar observations were found in a study by Kassir et al. (1989) on limonene, a major constituent of citrus essential oil isolated from abraded fresh peels of bitter orange *Citrus aurantium* L. against *Cx. quinquefasciatus* under laboratory conditions. Limonene was reported to demonstrate interference with oviposition as indicated by the zero egg rafts deposited in 1 and 50ppm compared with control water. All of the adults which were used in oviposition experiments in the respective study died after drowning within 2 weeks. Inspection of the water surface tension (by ring method) revealed that the tension was reduced in limonene-treated water respectively compared to control water.

The toxicity of the acethonilic *I. cairica* leaves extract may also be a possible factor for the mortality on gravid *Cx. quinquefasciatus* females during alighting in the treated oviposition site. Pre-oviposition drinking by *Culex* mosquitoes has been reported by Weber and Tipping (1993). It has been found that since drinking occurs before oviposition began, contact chemoreceptors on the mouthparts could be involved in the final stages of ovisite acceptance. Time spent in drinking exceeds that necessary for mere tasting, and drinking may also serve to distend the abdomen and assist in movement of eggs through the oviduct (Weber and Tipping 1990). Therefore, there is a possibility that this behavior may have caused poisonous interference in biochemical and
physiological functions of the gravid female mosquitoes in the current study due to the active compounds in the tested plant extract solution. The physiological constraints during oviposition might have caused less resource for egg deposition and eventually led gravid *Cx. quinquefasciatus* females to death.

The ability of acethonilic extract of *I. cairica* leaves to attract and kill gravid *Cx. quinquefasciatus* female mosquitoes can be very useful tool in mosquito control programs and hopefully this study can assist in further analysis on the interference caused by the tested plant extract on oviposition activity of *Cx. quinquefasciatus* mosquitoes.

On the whole, acethonilic extract of *I. cairica* leaves were found to effectively attract gravid *Cx quinquefasciatus* females and subsequently cause mortality of the gravid females, inhibiting egg deposition. The difference in color intensity also influences the oviposition site selection by the gravid females, whereby oviposition site with tested plant extract of darker color intensity attracts more gravid females. However, conclusion from this study was deduced from relatively short-run laboratory experiments, thus longer and detailed studies as well as field evaluations are required. Isolation and identification of specific phytochemicals responsible as oviposition attractant and further investigations on the factors causing mortality of the gravid females are required for better understanding on *I. cairica* plant extract interference in oviposition activity.

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REFERENCES


**Table 1**: Effects of different concentrations of acethonilic extract of *Ipomoea cairica* leaves extract on gravid *Culex quinquefasciatus* females mortality in single and multiple solutions tests.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>50ppm</th>
<th>150ppm</th>
<th>300ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>0.00±0.00a</td>
<td>1.00±0.58ab</td>
<td>2.00±0.58bc</td>
<td>3.67±0.33c</td>
</tr>
<tr>
<td>Multiple</td>
<td>0.00±0.00a</td>
<td>0.67±0.33a</td>
<td>4.00±0.58b</td>
<td>11.33±0.88c</td>
</tr>
</tbody>
</table>

Note: *At the horizontal line, the mean value followed by different letters are significantly different (Tukey test, P>0.05).*
Figure 1: Reflectance spectra of *Ipomoea cairica* plant extract at different concentrations of 50, 150 and 300 ppm.
Figure 2: Effects of visual stimuli on oviposition site selection by *Culex quinquefasciatus* gravid females.