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ASSESSMENT OF AMMONIUM-BASED SOLUTIONS (ABS) AS *Aedes aegypti* OVIPOSITION ATTRACTANT

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ABSTRACT

Aedes aegypti exhibit a higher preference for ovipositing their eggs in water that possess with dissolved mineral and nutrient for the survival of their progeny. These solutions usually contain ammonia which was formed due to decomposing process of decaying organic matter in the water. Therefore, the understanding of oviposition preferences is crucial for developing effective strategies in vector control programme. The attractiveness of aqueous ammonia, ammonium nitrate and a mixture of ammonium and acid lactic to gravid *Ae. aegypti* were accessed through dual and multiple choice bioassays under laboratory conditions. In dual and multiple choice bioassays, female mosquitoes was highly tend to oviposit their eggs in the mixture of ammonium and acid lactic solution (152.87±11.06) compared to the ammonium nitrate (134.13±10.32)) and ammonium aqueos (141.60±11.93). Solution of ammonium nitrate was less effective to attract gravid *Aedes* in dual bioassays' studies ($P>0.05$). This finding indicates that the combination of ammonium and acid lactic could be used in ovitrap surveillance programme. However, further research on combination of ammonium and acid lactic is warranted to find a better oviposition attractant for *Ae. aegypti.*

Keywords: *Aedes aegypti* oviposition, ammonium, aqueous ammonia, ammonia-based solution, ammonium nitrate, oviposition attractants

ABSTRAK

Aedes aegypti menunjukkan tahap kecenderungan yang tinggi untuk bertelur di dalam air yang mempunyai kandungan mineral serta nutrien untuk kelangsungan hidup progeni. Larutan ini biasanya mengandungi ammonia yang terbentuk daripada proses penguraian bahan organik serta bahan mendapan di dalam air. Oleh itu, pemahaman tentang keutamaan oviposisi adalah penting dalam membangunkan strategi yang lebih efektif di dalam program kawalan vektor. Daya tarikan terhadap larutan akues ammonia, ammonium nitrat dan campuran ammonium dan asid laktik terhadap gravid *Ae. aegypti* diuji dengan menggunakan ujian dwi bioasai dan pelbagai pilihan pada persekitaran makmal. Ujian bioasai terhadap dwi dan pelbagai pilihan menunjukkan kecenderungan yang tinggi untuk nyamuk betina bertelur di dalam campuran ammonium dan asid laktik (152.8 ± 11.06) berbanding ammonium nitrat (134.13 ± 10.32) dan akues ammonia(141.60 ± 11.93) Larutan ammonium nitrat adalah kurang efektif untuk menarik nyamuk *Aedes* gravid di dalam ujian dwi bioasai. Hasil penemuan ini menunjukkan gabungan ammonium dan asid laktik boleh digunakan dalam progam pemantauan ovitrap. Walau bagaimanapun, kajian lanjut terhadap campuran ammonium dan asid laktik adalah perlu bagi mendapatkan bahan penarik yang lebih baik terhadap nyamuk *Ae. aegypti*.

Katakunci: Oviposisi *Ae. aegypti,* ammonium, akues ammonia, larutan berasaskan amonia, amonnium nitrate, bahan penarik oviposisi

INTRODUCTION

Aedes aegypti is a primary vector, while *Ae. albopictus* serves as a secondary vector in the transmission of dengue fever. Approximately 5.2 million cases were reported in 2019 compared to 2.4 million in 2010 and 3.6 billion people are at risk of dengue infection in more than 128 countries (Brady et al. 2012; Tian et al. 2022). In order to manage the transmissions of dengue, various activities such as adulticide and larvicide were implemented to effectively reduce mosquito populations. However, several studies indicated that insecticide resistance has developed especially in dengue-endemic localities where an extensive amount of adulticide and larvicide were used (Aris 2019; Leong et al. 2019; Rajendran et al. 2021; Rasli et al. 2018) Alternatively, the lethal ovitrap and ovitrap surveillance were also implemented besides continuous efforts of larval inspection by health authorities to improve vector control activities. However, some of the ovitrap activities lack to attract mosquitoes due to the used of unsuitable lures or ineffective attractants (Ahmad-Azri et al. 2019).

Therefore, the utilization of baited mosquito traps to capture *Aedes* mosquitoes has increased considerably in other countries. The standard solutions such as hay infusion, *Anacardium occidentale* infusion (Santos et al. 2010), *Pinus palustris* infusion (Obenauer et al. 2010), NPK (nitrogen, phosphorus, and potassium) fertilizer solutions (Ahmad-Azri et al. 2019) and cigarette butt solution (Dieng et al. 2014) were among the organic oviposition attractants that have shown to attract mosquitoes' oviposition. Even though, there is a few factors associated with the *Aedes* mosquito oviposition preference such as larval density, food supplied and natural enemies, most of the previous studies suggesting that chemical cues still the main factor of habitat suitability for their offspring (Albeny-Simoes et al. 2014; Mosquera et al. 2023; Shragai et al. 2019).

Several studies found that field-collected water containing ammonia could attract *Aedes* mosquitoes to oviposit their eggs (Geier et al. 1999; Kim et al. 2021; Xie et al. 2019). Study conducted in Potim, Brazil revealed that public water tanks contain 2 ppm of ammonium nitrate, which was identified as one of the potential factors contributing to the attraction of *Ae. aegypti* oviposition (Marques et al. 2013). Study by Cahyati et al. (2017) demonstrated that the combination of 12.75 ppm ammonium and 1.20 ppm lactic acid in hay infusion was highly effective in attracting *Aedes* spp. mosquitoes for oviposition. Another study by Darriet & Corbel (2008) revealed that larval rearing water of *Ae. aegypti* contained 2.5 times increase in ammonium concentration compared to the control and a study by Walker (2016) found that *Aedes* breeding in tree holes and tires also contained low concentrations of ammonium (Darriet & Corbel 2008; Walker 2016). Therefore, this study aims to examine three ammonium-based solutions (ABS) which are known to be attractive to *Ae. aegypti* for their ability to improve *Aedes* mosquito trap collections in mosquito traps.

MATERIALS AND METHODS

Mosquito Cultures

The susceptible strain of *Ae. aegypti* eggs used in this study were supplied by Institute for Medical Research (IMR). Mosquito larvae were maintained under controlled condition with 26±4℃ and 60±20% of RH. Both five-day-old male and females of mosquitoes were place in a holding cage for two hours to facilitate mating. Males were fed on 10% sugar solution *ad libitum* while females were fed on cattle blood until fully engorged through an artificial blood-feeding system using the Digital Thermo Blood Feeder (UKM Holdings, Sdn Bhd) for two hours (Yatim et al. 2021). The fully engorged mosquitoes was based on the expanded abdominal pleurites in mosquitoes. Ten females (two days post blood feeding) were used in each cage of the study.

Test Procedures

Lure selection

Ammonia solution, 30%, A.R., ammonium nitrate, A.R./ACS., and lactic acid, 88%, A.R., which is a commercial solution were purchased from R&M Chemicals, Malaysia. Stock solution was prepared from the material and the combination of each solution were mixed with distilled water to achieve desirable concentrations. We evaluated three potential ABS which has been successfully used in other oviposition studies to attract gravid mosquitoes. Solution A consist of a 1 ppm aqueous ammonia that was showed an attractive effect to *Aedes* mosquitoes (unpublished data). Solution B contains 2 ppm of ammonium nitrate, derived from a study conducted in Potim, Brazil. The concentration of ammonium nitrate was found to influence the choice of mosquito oviposition (Marques et al. 2013). As for the Solution C, it is a combination of 12.75 ppm ammonium with 1.20 ppm lactic acid which were previously identified as attractant in hay infusion and used in lethal ovitraps (Cahyati et al. 2017).

Evaluation of mosquito to ABS solution

The evaluation of the mosquito preference oviposition was performed using two assays; (i) dual-choice assays that each cage was offered one ABS with dechlorinated water (control) and (ii) multiple-choice assay in which three ABS solutions with one water were offered in the cage. No-choice assay also been conducted in which mosquitoes was offered four ovitrap of water (Mohd Ngesom et al. 2021; Satho et al. 2015). Mosquitoes distributed all eggs equally in the four ovitrap showing no bias between the corner of the cage.

A dual-choice assays was performed with 10 females *Ae. aegypti* were released into each cage and left undisturbed. After 48 h of the bloodmeal, two ovitrap containers were introduced and arranged diagonally opposite to each other in the corner (Figure 1a). Each ovitraps were ¾ filled with dechlorinated water and ABS solutions with a cone-shaped filter paper was placed on top of the ovitrap to serves as a suitable place for female *Ae. aegypti* oviposition (Figure 1b). In the multiple-choice assays, the preference of mosquitoes to oviposit their eggs were assessed using the i) Solution A, ii) Solution B and iii) Solution C, while one ovitrap was the dechlorinated water as a control. All the treatment and control containers in dual-choice and multi-choice bioassays were switched in every other position in each replicate to control for position bias (Figure 1c).

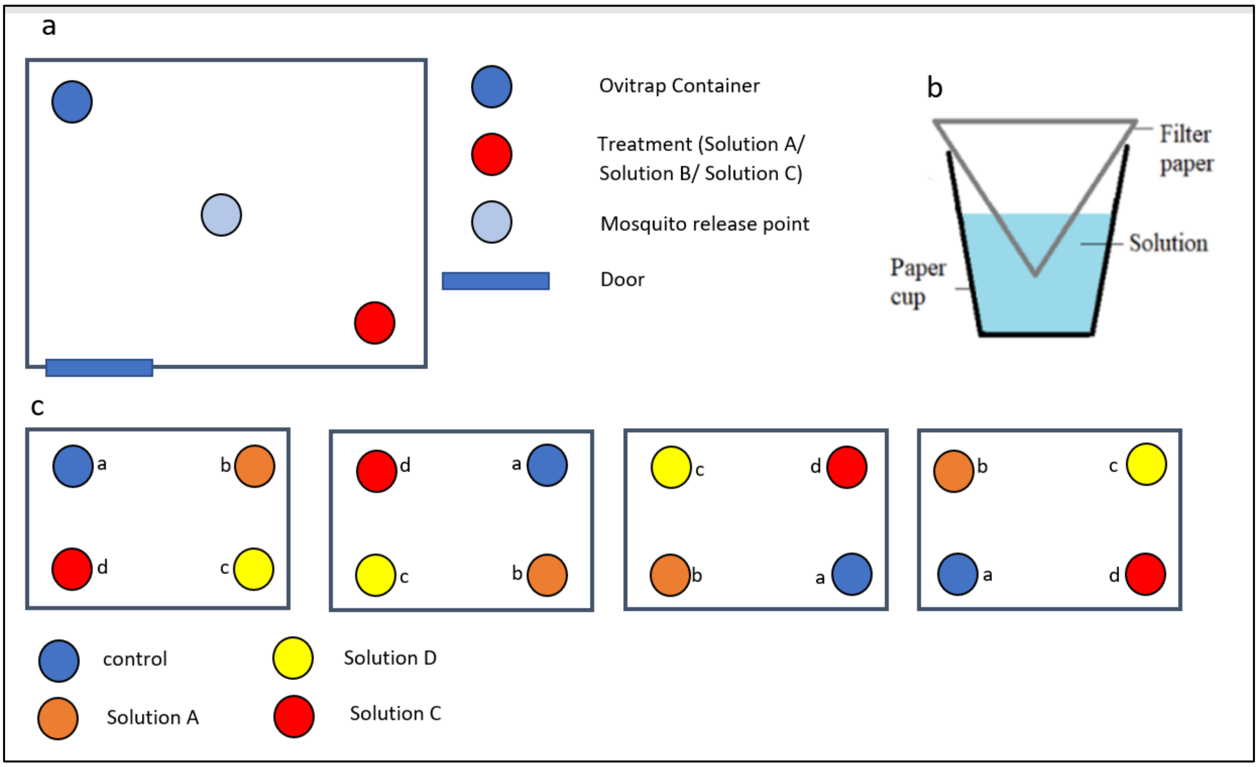

Figure 1. Oviposition bioassay design. a) dual-choice bioassay, b) ovitrap with filter paper and solution and, c) multiple-choice bioassays

Data Analysis

Data analyses were performed by using Statistical Package for Social Sciences (SPSS) Version 23.0. The number of eggs were counted and recorded. Each study was replicated 30 times for each replicate. All the data were tested for normality distribution using the Shapiro-Wilk test. For dual-choice experiments, the different number of eggs collected from different ovitrap were compared using a t-test with a statistically significant determined using Tukey methods with $P>0.05$. Additionally, the comparison of multiple-choice assays was performed using one-way ANOVA, followed by a Tukey post hoc test.

RESULTS AND DISCUSSION

In dual-binary assay, we observed that all of the ovitrap containers were visited by gravid *Ae. aegypti*, with the highest percentage numbers of egg collected in Solution C (152.87 ± 11.06, 55.26%) followed Solution A (141.60 ± 11.93, 53.71%) and Solution B (134.13 ± 10.32, 53.60%). Generally, Solution B treatment was less effective to attract gravid *Aedes* than Solution C and Solution A with no significant difference among water with Solution A ($P=0.249$), Solution B ($P=0.075$) and Solution C ($P=0.226$). (Figure 2).

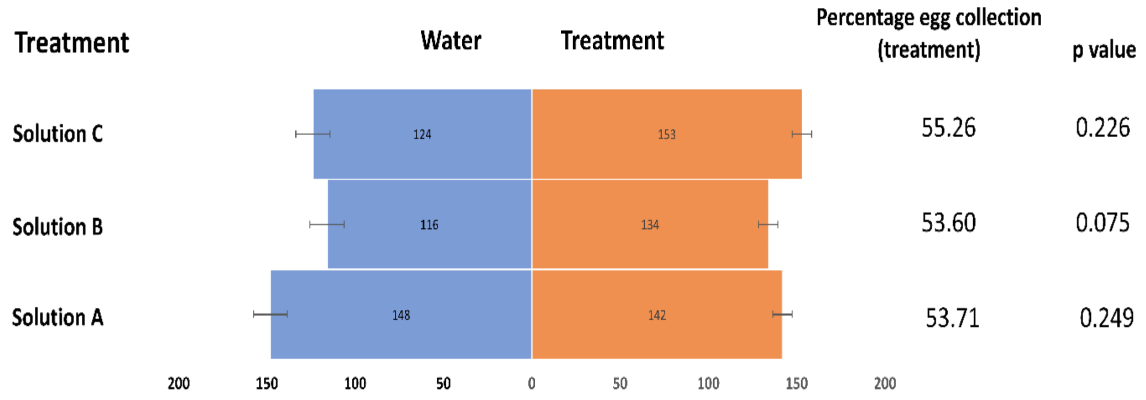

Figure 2. Response of female *Ae. aegypti* in a dual-choice assay

Meanwhile, in the multiple-choice assays, *Ae.aegypti* showed a strong preference for oviposition in Solution C and Control. A total of 15232 eggs were laid in all the cups, with 25% of the eggs in the ovitrap with Solution C and 23% in the control ovitraps. Additionally there was a significant difference among the eggs collected in each ovitraps (ANOVA, $F_{3,116}$= 2.864, $P=0.040$) (Figure 3).

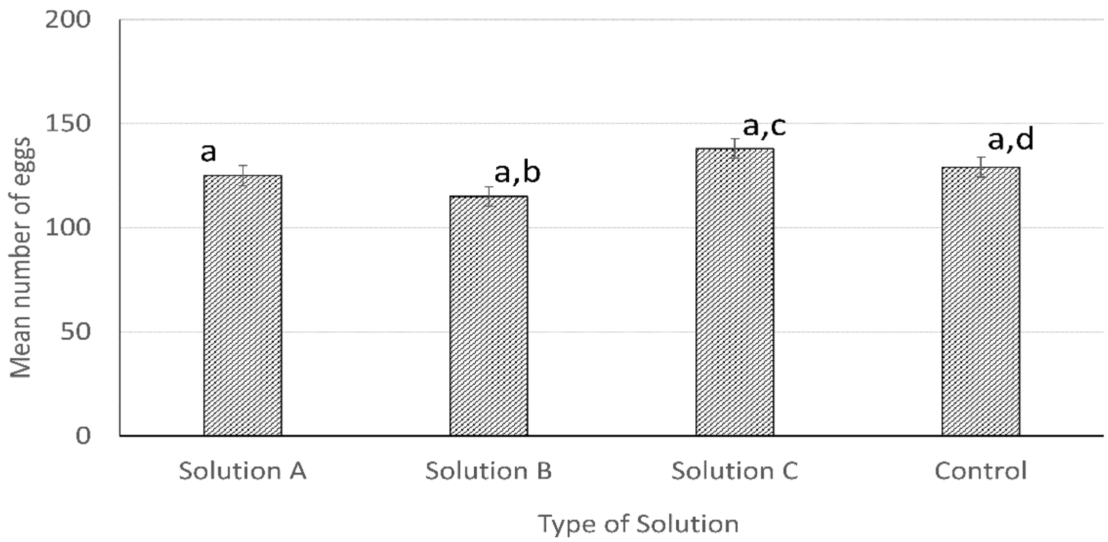

Figure 3. The preference of gravid oviposition in multiple-choice bioassays with different type of solutions. Tukey's *post-hoc* shown with different letter are statistically different from each other ($P<0.05$)

This study has explored the hypothesis of gravid females that attracted to the ammonia in the water suggests that the semiochemical may function as one of the important roles in mosquito oviposition. Semiochemical may help gravid *Aedes* mosquitoes to find any cryptic or suitable site to lay their eggs (Wooding et al. 2020). Furthermore, previous studies indicated that mosquitoes prefer to oviposit their eggs in the presence of decaying leaf with any other nutrient in water (Day 2016). Onchuru found that *Ae. aegypti* prefers to oviposit in fertilizer-enriched water that contains a low concentration of ammonium and sulphate (Onchuru et al. 2016). Further analysis revealed that this fertilizer-enriched water contained *Proteobacteria*, *Bacteroidetes* and *Firmicutes*. This shows that besides ammonium and sulphate, certain bacteria species could attract *Aedes* mosquitoes to oviposit in fertilizer-enriched water. Findings by Arbaoui & Chua (2014) demonstrated a significant impact on eggs collection using bacteria isolated from bamboo leaf infusion compared to a control medium with OAI value of +0.92 (Arbaoui et al. 2014).

Our findings indicated that the Solution C, which is found in hay infusion exhibited with the highest oviposition percentage during dual and multiple-choice bioassays. Similar results were recorded in 15% insulin plant infusion (*Costa igneus*) with 475.3 compared to the control (Iyyappan et al. 2022). Shu and Shelomi (2021) also demonstrated the fermented leaf infusion significantly increases the collection of the traps and boosts the egg hatching rates. In another study, *Ae. albopictus* and *Ae. aegypti* were observed to show a significant attraction towards senescent bamboo (*Arundinaria gigantea*) and white oak (*Quercus alba*) compared to other compounds tested (Ponnusamy et al. 2010). Moreover, multiple studies have been conducted on mosquito oviposition preferences with other fermented plant infusion and showed a promising result (Nascimento et al. 2020, Ong et al. 2020, Suman 2019).

Several studies have identified that carboxylic acids, 1-octen-3-ol and L-lactic acid and saturated aldehydes as an attractive human cue for mosquito species (Hinze et al. 2021), Refining the combination of ammonia with other compound such as acid lactic (Geier et al. 1999), nitrate, ketone (Mahadevan & Sen 2017), carboxylic acid (Smallegange et al. 2011), hexanoic acid (Krockel et al. 2006) may increase the attractant effect on gravid female mosquitoes. It is noteworthy that ammonia is not as powerful as hay infusion, thus, combination of ammonia with lactic acid considered as internal standard odor (Smallegange et al. 2011). Further studies under semi and field environment are essential to determine the role of each chemical composition of attraction for Aedes spp. mosquitoes that can be employed in vector programme strategies.

CONCLUSION

In general, the combination of ammonium and acid lactic had more attractive effects than single aqueous ammonia and ammonium nitrate. Further studies are warranted to understand and investigate the combination of ammonium with acid lactic is also effective in other settings such as semi field and field conditions towards a new development of attractant against *Ae. aegypti.*

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AUTHORS DECLARATIONS

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Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Declarations
This study did not use laboratory animals or humans for mosquito blood feeding. The blood feed using cattle blood was ethically approved by the Animal Ethics Committee from National University of Malaysia, with UKMAEC approval number: PARST/PP/2017/AISHAH/22-NOV./885.-NOV-MAC.-2019-AR-CAT2. The study was conducted with the permission of the Medical Research Committee at the Faculty of Medicine, National University of Malaysia, Under project: FF-2017-492.

Data Availability Statement
Not applicable

Authors' Contributions
Mohd Farihan Md Yatim: Conceptual, conducting research, and writing the draft manuscript; Ahmad Mohiddin Mohd Ngesom: Data analysis, reviewing and editing the manuscript; Faizul Akmal Abdul Latif: Reviewing and editing the manuscript; Mohd Amierul Fikri Mahmud: Reviewing and editing manuscript; As'malia Md Lasim: Data analysis, reviewing and editing the manuscript: Aishah Hani Azil: Designing the experiment, research supervision, reviewing and editing the manuscript.

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