scientific reports

OPEN

Effect of different lining paper materials and infusions on oviposition preference of *Aedes aegypti* (Diptera: Culicidae) gravid mosquitoes

Mahfuza Momen, Kajla Seheli, Md. Aftab Hossain, Ananna Ghosh & Md. Forhad Hossain

***Aedes aegypti* (Linnaeus) mosquito is a vector responsible for increasing global public health concerns due to its rapid geographical spread and increasing vectorial capacity. Understanding the breeding behavior of *Ae. aegypti* is crucial to control this vector and thereby limiting the spread of diseases. To study this mosquito breeding in the laboratories and ovitraps, different types of lining-papers, surrounding inner surface of a container along with different types of added infusions, are used. Lining-papers serve as surfaces for oviposition and infusions provide the essential environment for nurturing. The types of oviposition surfaces and infusions used in laboratory rearing facilities or ovitraps influence oviposition preference, changing the breeding behavior of *Ae. aegypti* gravid mosquitoes. This study presents a comparative study on oviposition preferences for six different types of lining-papers and six different types of infusions in a bioassay cage. ANOVA analysis shows a significant effect on oviposition preference of different types of lining-papers that served as oviposition surfaces. The highest oviposition activity was observed for the '*agri seed germination paper-75*' lining-paper when normal tap water infusion was used. Likewise, statistical analysis shows that when a '*plain printing offset paper 80 GSM*' lining-paper was used, a highly statistically significant effect on oviposition preference is observed for different types of infusions used in the oviposition cups.**

Keywords Yellow fever mosquito, *Aedes*, Oviposition, Infusion, Surface

Abbreviations

Ae.	*Aedes*
GSM	Grams per square meter
ECDC	European Centre for Disease Prevention and Control
IPM	Integrated pest management
RH	Relative humidity
OAI	Oviposition activity index
LSD	Least significant difference

Aedes aegypti (Linnaeus) female mosquitoes exhibit anthropophilic behavior and humans are their favorite supply of blood[1,2]. It is one of the most prominent mosquito vectors responsible for transmitting viruses such as dengue, chikungunya, and Zika[3]. The magnitude of disease transmission by this vector raised serious health concerns. Since the start of 2023, five million confirmed cases and more than five thousand fatalities associated with dengue fever, reported across 80 countries in different regions of the World (Africa, Americas, South-East Asia, Western Pacific, and Eastern Mediterranean Regions)[4]. A similar report was published by the European Centre for Disease Prevention and Control (ECDC) on 30 September 2023 for chikungunya. This report has revealed that the chikungunya virus infected about 0.44 million people between October 2022 and September 2023, with 350 deaths documented worldwide[5].

Ae. aegypti thrives in urban and suburban environments due to the abundant presence of diverse oviposition sites that facilitate the deposition of eggs by this mosquito species[6,7]. For this reason, *Ae. aegypti* mosquitoes are

Insect Biotechnology Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission, Dhaka, Bangladesh. email: mahfuza37@baec.gov.bd

capable of spreading vector-borne diseases rapidly. The principal strategy for the management of vector-borne diseases of this characteristic involves effective vector control. However, controlling this mosquito species is extremely difficult because of its remarkable adaptive capacity and ability to deposit eggs in diverse locations, many of which are difficult to access[8]. In-depth understanding of the behavioral and breeding habits of *Ae. aegypti* is essential to control this vector by implementing an integrated pest management (IPM) approach where physical, biological, and chemical strategies can be used altogether[9].

All mosquitoes undergo four unique stages throughout their life cycle: egg, larva, pupa, and adult (see Fig. 1)[10]. After consuming enough blood, gravid mosquitoes select a suitable oviposition site to lay their eggs[11]. *Ae. aegypti* gravid mosquitoes usually deposit their eggs on a surface around stagnant freshwater storage[11]. Water is essential for mosquito breeding because mosquito eggs require some form of water to hatch. Mosquitoes use their hygro sensory system to detect humidity cues in order to explore their surroundings successfully in search of an appropriate oviposition site[12]. After coming into direct contact with water, the female mosquitoes use their sensory and gustatory apparatus to detect the quality and texture of oviposition surfaces[13,14]. A variety of suitable oviposition sites, such as old tires, clogged drains, canvas and plastic sheeting, and other stagnant water containers produced by human activities, are available for deposition of eggs[11]. The eggs that have been laid have the ability to live for extended periods[15]. The eggs undergo hatching upon submergence in stagnant water. After

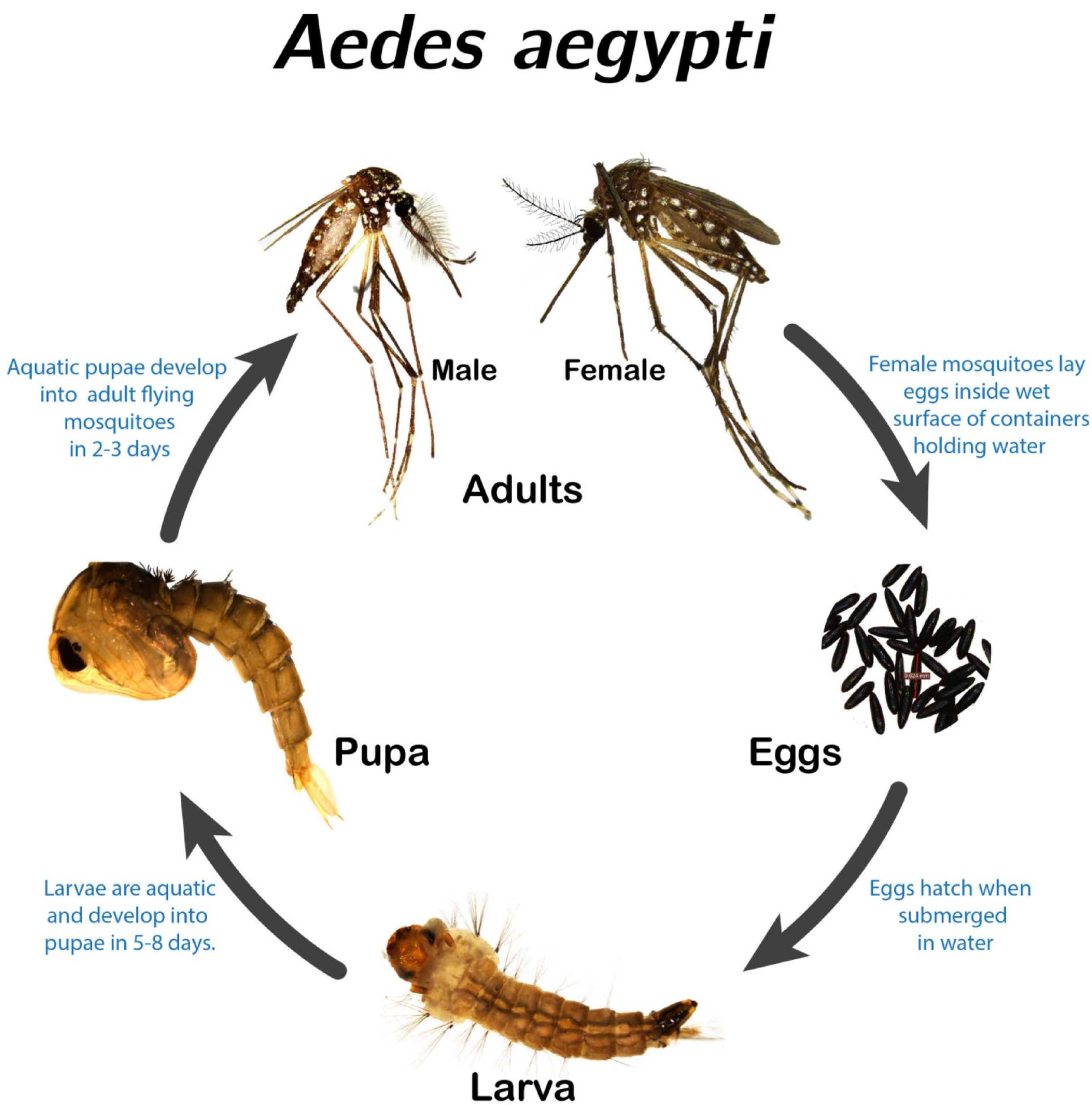

Fig. 1. The entire life cycle of *Aedes aegypti*: egg, larva, pupa, and adult. This mosquito has an aquatic larva and a non-feeding pupa stage in its life cycle. The aquatic pupae are transformed into active flying adult mosquitoes at the final stage.

hatching of eggs, the larvae feed on algae and other organic matters present in water[16]. Next, the larvae transform into pupae which also reside in water until they emerge as adult mosquitoes.

The egg stage is the most sensitive to environmental conditions in the first hours after being deposited[17]. For this reason, *Ae. aegypti* gravid mosquitoes show unique skip-oviposition behavioral pattern to ensure the maximum survival of the offsprings by distributing eggs in multiple suitable water containers[18]. The selection of oviposition sites by a female mosquito is a conscious and analytical process, influenced by various physical and chemical factors present on an oviposition surface and in the water contents of a container[19]. Water quality and contents in the water (i.e., presence of chemicals, microorganisms, etc.) of a container directly impact the aquatic larval and pupal stages of their life cycle till the adult stage. Therefore, in order to select an oviposition location, a gravid mosquito first assesses the nutritional quality of water as well as the likelihood of survival of their offsprings through visual and chemical cues[11]. In addition, the availability of larval food and the presence of previous mosquito eggs or larvae influence the selection of oviposition sites[19–21].

Eggs of *Ae. aegypti* absorb water in the early hours after egg deposition to facilitate embryogenesis. Serosal cuticle formation is completed after water absorption[22]. For this reason, a proper moisture level of an oviposition surface is essential for freshly laid eggs in the first hour to form a darker dehydration-resistant protective cuticle[23]. As demonstrated in earlier research works, female mosquitoes exhibit a preference for a moist surface that is suitable for the survival and development of their eggs[11,24]. In addition to the moisture level, gravid mosquitoes also consider the moisture absorbance capacity, temperature, surface texture, heat resistance capacity as well as the presence and absence of chemical substances and microorganisms in the damp surface of an egg-laying container[23–25].

In the bioassay cages and ovitraps (container breeding of *Aedes* spp.), various types of suitable lining-papers are used. The lining-papers can mimic the natural container inner surfaces of the preferred breeding sites of mosquitoes. However, there is a lack of comprehensive research on the effect of different types of inner surface lining-papers on oviposition site selection and oviposition preference[23–25]. Furthermore, no comparative study was found that revealed the effect of different infusion and lining-paper combinations on oviposition preference.

Microorganisms are an essential food source for mosquito larvae[26]. To mimic the natural breeding environment in the laboratory mosquito rearing facilities and ovitraps surveillance, occasionally a lab-made infusion containing organic substances is added to water-filled containers where mosquitoes deposit eggs[27–29]. The infusions help to sustain the growth of microorganisms and serve as a food source for mosquito larvae. Artificially made infusions can be formulated from a variety of sources, including hay, grass, leaves, fruits, different plant extracts and even animal blood. Previous studies revealed that the type of infusions affects the oviposition behavior of gravid mosquitoes. For example, previous studies revealed that various semiochemicals and different plant organic infusions influenced oviposition preference and these infusions could be used to manipulate the skip-oviposition behavior (dispersing of eggs from a single batch across multiple oviposition sites instead of depositing all eggs in a single location) of gravid mosquitoes[19,30,31].

However, there is a continuous search for more efficient and effective infusions that can be used in the containers of a bioassay cage and ovitraps to attract *Ae. aegypti* gravid mosquitoes for oviposition. Previous studies revealed that infusions comprising of larval rearing water and pre-existing larvae (conspecific and heterospecific) hold promise for a more sustainable, simple and cost-effective solution that is more specific for container breeders like *Ae. aegypti*[27,32,33]. However, limited information is available regarding these infusions containing larval-rearing water. Further investigation is required to reveal the influence and effectiveness of these infusions comprised of larval-rearing water on oviposition preference.

In this work, a comparative study on the effect of different types of oviposition surfaces and infusions on oviposition preference was carried out. First, the effect of six different types of lining-papers used as oviposition surfaces in combination with the *normal tap water* infusion was investigated. Furthermore, the effect of six different types of infusions in combination with the *'plain printing offset paper 80 GSM'* lining-paper was investigated. Our statistical analysis showed that different types of lining-papers and infusions significantly influence the oviposition preference of *Ae. aegypti* gravid mosquitoes.

Material and methods

Laboratory rearing of *Aedes aegypti* mosquitoes

In this study, oviposition preference was investigated by counting eggs collected from a laboratory-maintained *Ae. aegypti* colony (see the workflow diagram in Fig. 2). In the laboratory, relative humidity, temperature, and light–dark cycle were consistently maintained at 60–80% RH, 27 °C (± 2 °C), and 12hL:12hD respectively.

In the first step, mosquito eggs were collected from a laboratory-maintained *Ae. aegypti* colony (see Fig. 3) and transferred to a hatching tray containing water to keep the eggs wet in such a way that the lower part of the egg collecting strips were immersed in water. The newly hatched first instar larvae that emerged within 6 h were collected using a dropper and placed into a rearing tray.

The larvae (see Fig. 3b) were maintained in a larval rearing tray (40 cm × 27.30 cm) at a larval density of 1000 per tray containing 1000 ml water. Sufficient water was added from time to time to maintain the same water level during the larval rearing period. Daily 0.70 g of fish feed (aquarium fish food Super Nova manufactured by Perfect Companion Group Co., Ltd. Thailand) was provided for 1000 larvae per tray. The rearing trays were cleaned periodically to remove excess waste materials using vacuum aspiration method (with the help of a vacuum pump). Once pupation was started, all the pupae were collected from the tray. The newly emerged pupae were collected, kept in plastic vials, and observed for adult eclosion.

A total of 40 adult mosquitoes (20 females and 20 males) at a 1:1 sex ratio was kept in a cage (35 cm × 28 cm × 20 cm size). Adult mosquitoes were provided with a daily supply of food using a fresh cotton ball soaked in a 10% glucose solution for the purpose of nourishing the adults. Furthermore, 5 days old female mosquitoes were fed chicken blood by artificial membrane feeding (see Fig. 3c,d), following a modification

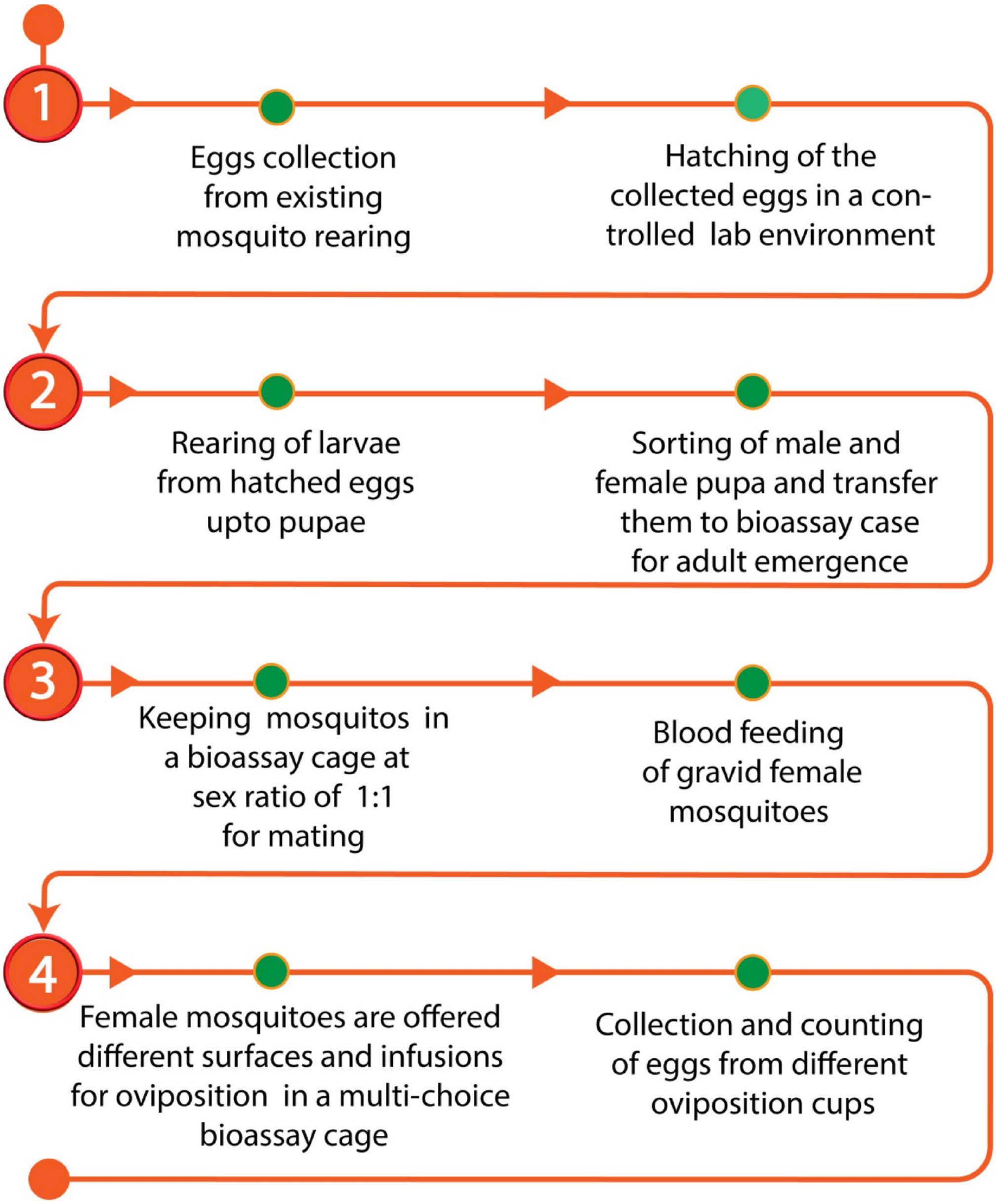

Fig. 2. Steps for the experimental workflow. Our experiments started with collecting fresh eggs from an existing mosquito-rearing laboratory. To observe oviposition preference of gravid mosquitoes, eggs were collected from different oviposition cups containing different oviposition lining materials and infusions.

of the technique of Mourya et al.[34]. In this process, a blood bag containing anticoagulant (citrate phosphate dextrose) was used to collect chicken blood from the slaughterhouse of the local market. Small petri plates were filled with about 20 ml of blood (see Fig. 3c,d) and then turned upside down on top of adult mosquito cages. Each petri plate was covered by a bag filled with warm water (around 37 °C) to keep the blood warm. Blood feeding was conducted daily at about 3:30 PM. Female mosquitoes were allowed to blood feed for 30–40 min each day for two days. After three days of being blood-fed, the females started laying eggs.

To study the effect of different lining-papers that functioned as oviposition surfaces, six types of papers were used as inner surface lining on the oviposition cups (breeding container) and '*normal tap water*' infusion was added. Similarly, to study the effect of different infusions, six types of infusions were added for each oviposition cup and '*plain printing offset paper 80 GSM*' was used as a lining-paper that functioned as oviposition surface.

To investigate oviposition preference of gravid females, a bioassay cage for a multi-choice test was used (see Fig. 4). Six oviposition cups (200 mL) made of clear plastic (Polystyrene) were placed in the bioassay cage. Inner surfaces of the cups were covered using different lining papers (see Table 1) which were functioned as oviposition surfaces.

We obtained those six types of lining papers from different manufacturers for our study and identified them by their respective commercial names. Details of lining papers with their respective code name used in this

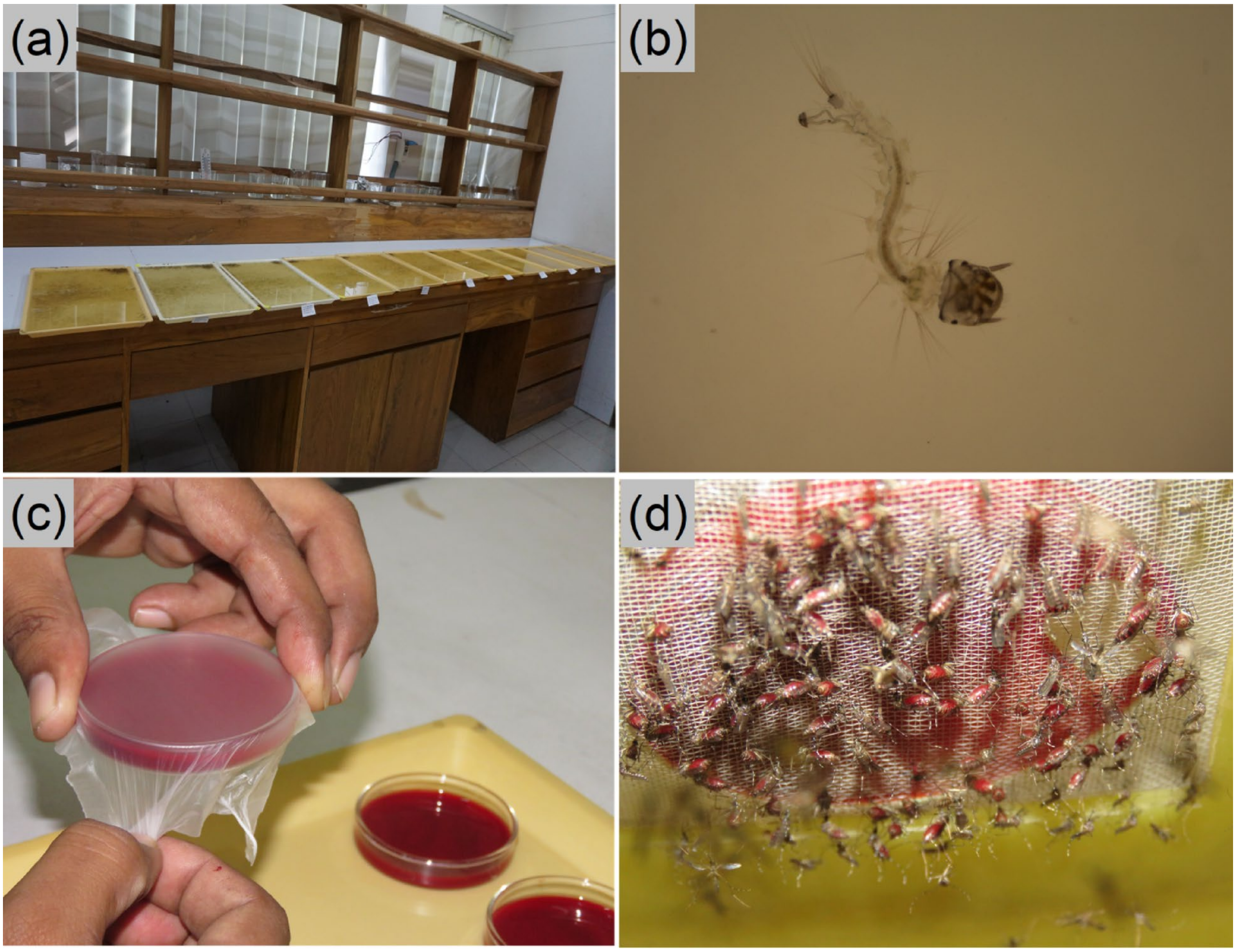

Fig. 3. *Aedes* spp. rearing process (**a**) colony rearing room (**b**) larval stage (**c**) an artificial membrane blood-feeder setup preparation (**d**) appearance of female *Aedes aegypti* mosquitoes that have engorged.

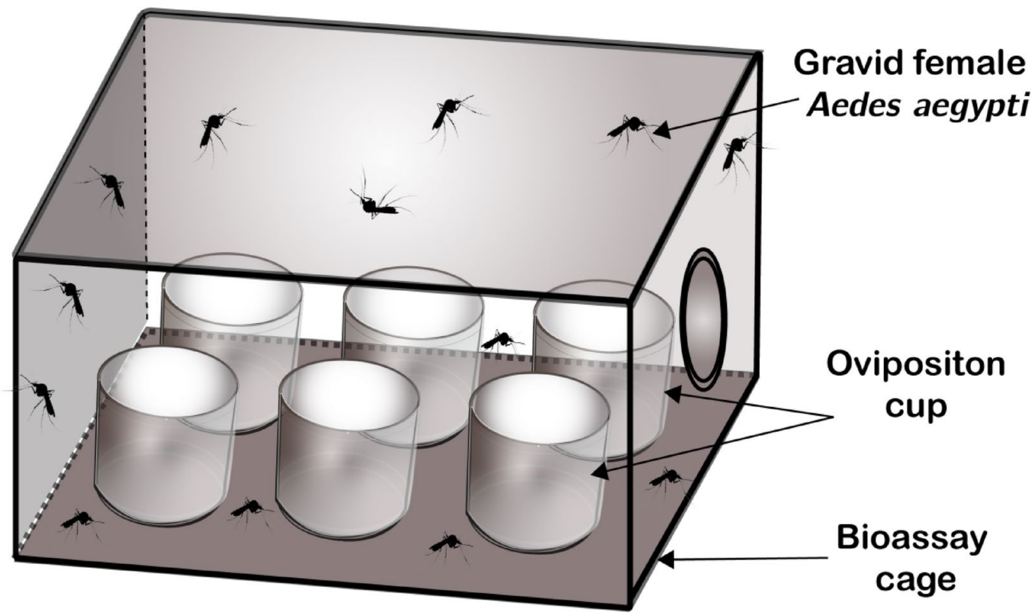

Fig. 4. Schematic diagram of a bioassay cage for a multi-choice test for investigating oviposition preference.

Lining-paper (code name)	Type of paper and manufacturer	Grams per square meter (GSM)	Thickness (µm)	Texture	Average surface roughness, Ra (µm)	Color
OS-A	Plain printing offset paper 80 GSM (Bashundhara Paper Mills Limited, Bangladesh)	80	92.5 ± 2.52	Plain	2.91	White
OS-B	Qualitative filter paper (Whatman®)	87	180 ± 0.95	Plain	9.08	White
OS-C	Agri seed germination paper KK030 (Kalpkala Paper & Pulp Paper Industries, India)	160	374.4 ± 51.56	Crepe	60.62	White
OS-D	Agri seed germination paper KK029/85 (Kalpkala Paper & Pulp Paper Industries, India)	84	176 ± 56.71	Crepe	43.46	White
OS-E	Agri seed germination paper-125 (Kalpkala Paper & Pulp Paper Industries, India)	130	262.88 ± 48.58	Crepe	97.90	Light shade of brown
OS-F	Agri seed germination paper-75 (Kalpkala Paper & Pulp Paper Industries, India)	79	156.29 ± 58.45	Crepe	57.21	Warm and earthy shade of brown

Table 1. Properties of different types of lining-papers and their code name used in this experiment.

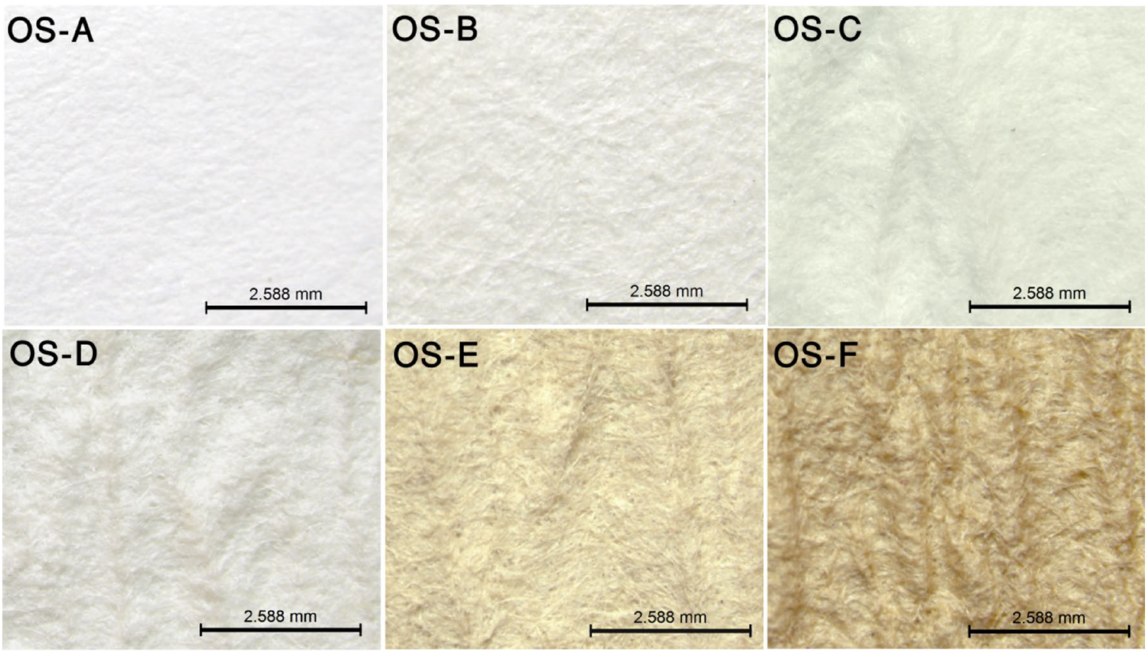

Fig. 5. Images of different lining paper surfaces: OS-A (plain printing offset paper 80 GSM); OS-B (qualitative filter paper); OS-C (agri seed germination paper KK030); OS-D (agri seed germination paper KK029/85); OS-E (agri seed germination paper-125); OS-F (agri seed germination paper-75).

article is presented in Table 1. Images of the surfaces of the lining papers are also shown in Fig. 5. In addition, surface profiles of the lining papers were investigated by a stylus profilometer (Dektak 150 Surface Profiler, Veeco Instruments Inc.) as shown in Fig. 6. A lateral scan of 5000 µm was performed using a stylus of radius 12.5 µm (force 1.0 mg) for each lining paper. From the surface profiles, average roughness R_a (see Table 1) of each lining paper were calculated using the following equation:

$$R_a = \frac{1}{L}\int_{x=0}^{x=L} |y|\, dx$$

Here, y is the surface height and R_a is the arithmetic average deviation from the mean line within the lateral scan length (L) in the x direction.

Table 2 presents the six different types of infusions with their code name used in this article. Please note that the *normal tap water (IN-B)* is also termed as infusion in this article. As *Ae. aegypti* females do not lay eggs in the absence of a wet surface[23], therefore oviposition cups with the *normal tap water (IN-B)* and the *plain printing offset paper 80 GSM (OS-A)* lining were considered as control in this study. In addition, the *OS-A* lining paper and *IN-B* infusion were used for regular colony maintenance of *Ae. aegypti*.

This study was divided into two separate experiments. In the first experiment, three days after blood feeding the gravid mosquitoes were given a choice of six oviposition cups to lay eggs where different types of lining-

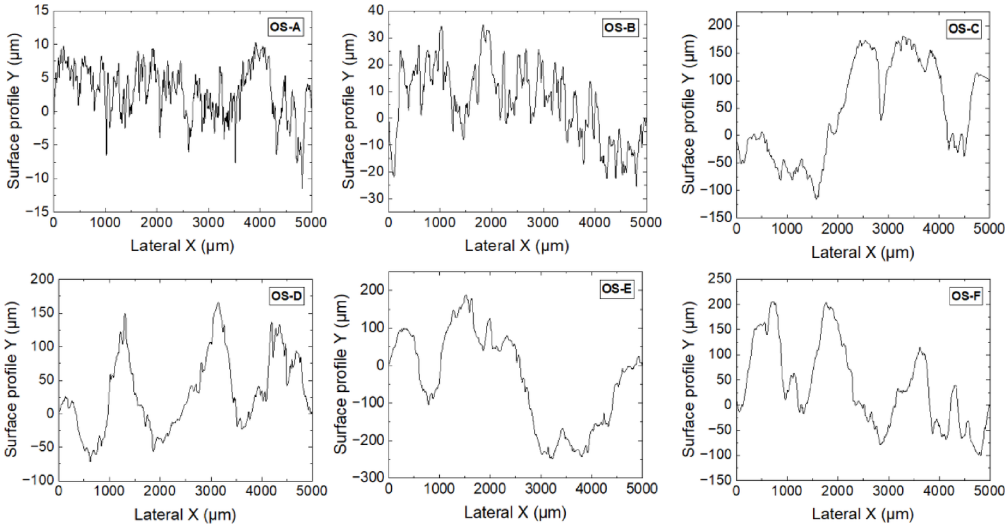

Fig. 6. Surface profile of different types of lining papers: OS-A (plain printing offset paper 80 GSM); OS-B (qualitative filter paper); OS-C (agri seed germination paper KK030); OS-D (agri seed germination paper KK029/85); OS-E (agri seed germination paper-125); OS-F (agri seed germination paper-75).

Infusion (code name)	Type of infusion
IN-A	saline water (salinity 35 ppt)
IN-B	normal tap water
IN-C	only larval diet in water
IN-D	larval-rearing water
IN-E	larval-rearing water with *Aedes albopictus* second instar larvae
IN-F	larval-rearing water with *Aedes aegypti* second instar larvae

Table 2. Different types of infusions and their code names were used in this experiment.

Experiment 1: Effect of different oviposition surfaces		Experiment 2: Effect of different infusions	
Lining-paper (code name)	Type of infusion (code name)	Infusion (code name)	Lining-paper (code name)
OS-A	IN-B	IN-A	OS-A
OS-B	IN-B	IN-B	OS-A
OS-C	IN-B	IN-C	OS-A
OS-D	IN-B	IN-D	OS-A
OS-E	IN-B	IN-E	OS-A
OS-F	IN-B	IN-F	OS-A

Table 3. Combination of different types of infusions and lining-papers used to observe the effect on oviposition preference.

papers along with *normal tap water (IN-B)* were used in each oviposition cup (see Table 3). Similarly, in the second experiment, *Ae. aegypti* gravid mosquitoes were given a choice to lay eggs between six oviposition cups where different infusions were added in each oviposition cup along with *plain printing offset paper 80 GSM (OS-A)* (see Table 3). We selected *plain printing offset paper 80 GSM (OS-A)* for the second experiment because it was already part of our standard laboratory rearing process. Additionally, the smooth and white surface of this paper facilitated accurate egg counting. Twenty-four hours prior to the experiment, larval rearing water of *Aedes aegypti* was prepared as previously described and stored at 27 ± 1 °C temperature. Shortly before pupation, larvae were removed from the rearing water using filter paper for filtration to prepare the larval water infusion. To examine the oviposition responses of gravid *Ae. aegypti* females to pre-existing larvae, equal numbers (n = 20) of second-instar *Ae. albopictus* and *Ae. aegypti* larvae were added to oviposition cups with infusion *larval rearing water with Ae. albopictus larvae (IN-E)* and *larval rearing water with Aedes aegypti larvae (IN-F)*, respectively. Each oviposition cup is provided with an equal amount of infusion. After three days of oviposition, each lining-paper was collected from the oviposition cups to count the number of eggs. Each experiment was replicated three times, with oviposition cups randomly repositioned to different locations within the cages for each repetition.

Data analysis

The effect of different oviposition surfaces and water composition was expressed by the oviposition activity index (OAI) as described by Kramer and Mulla[35]. The oviposition activity index (OAI) was calculated by the following formula:

$$OAI = (N_t - N_c) / (N_t + N_c)$$

where, N_t represents the number of eggs observed on the lining-material (oviposition surface) of the oviposition cups, and N_c represents the number of eggs observed on the lining-material (oviposition surface) of the control oviposition cup.

The oviposition activity index (OAI) is bounded within the interval of + 1 to − 1, where 0 indicates no response. A positive value indicates that the infusion or lining material of an oviposition cup functions as an attractant, whereas a negative value indicates that the infusion or surface has a deterrent effect.

One-way analysis of variance (ANOVA) was used to compare the influence of the six different types of oviposition surfaces (lining-papers) as well as six different types of infusions. The Fisher's Least Significant Difference (LSD) test was used to compare the different oviposition surfaces as well as different infusions. In this context, $P \leq 0.05$ was referred to as statistically significant and $P \leq 0.001$ was referred to as statistically highly significant. The statistical analyses were carried out by OriginPro® software.

Results

Oviposition preference for different types of paper

In the experiment of oviposition preference for different lining-paper surfaces, the *plain printing offset paper 80 GSM (OS-A)* lining-paper attached to the inner surface of the oviposition cups was considered as control (see Table 1).

Overall, the mean number of eggs laid in the oviposition cups by the gravid mosquitoes varied depending on the types of lining-papers functioned as oviposition surface (see Fig. 7a). Statistical analysis showed statistically significant (F = 4.852; df = 5, 12; $P = 0.01$) evidence of correlation of oviposition preference with different lining-papers (see Fig. 7b). The highest amount of oviposition activity (see Fig. 8) was observed in the case of the *agri seed germination paper-75 (OS-F)*. According to mean comparison conducted in pairs using the Fisher's Least Significant Difference (LSD) test, only the effect of *agri seed germination paper-75 (OS-F)* paper lining was significantly different from other five types of lining-paper.

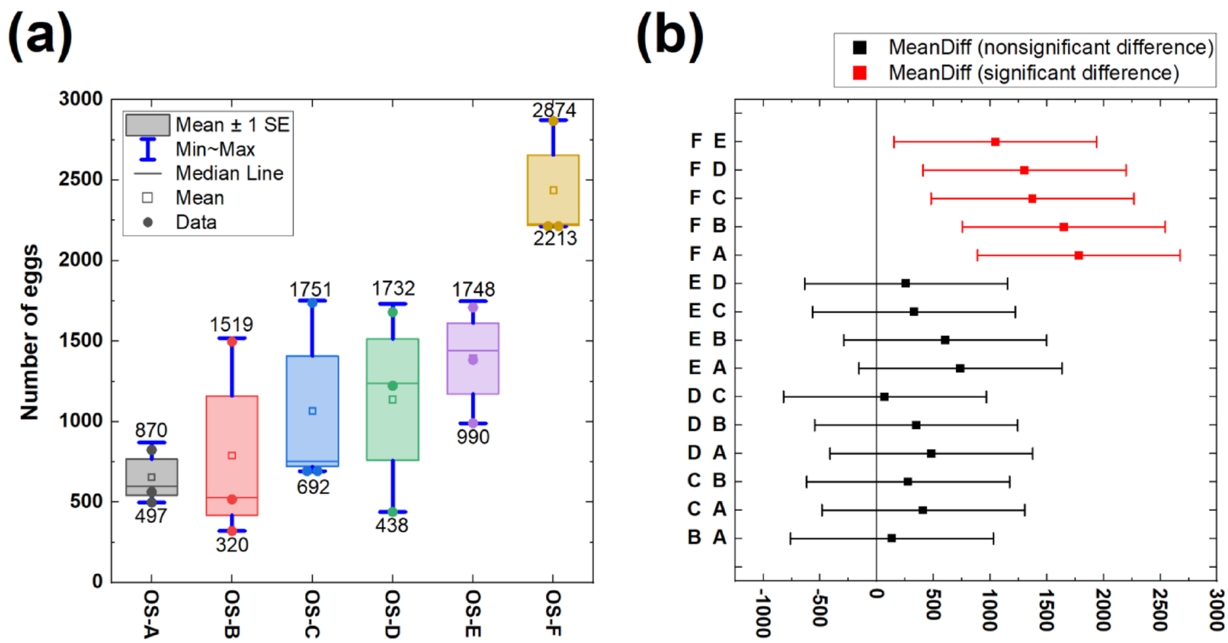

Fig. 7. Oviposition preference of *Aedes aegypti* gravid mosquitoes in response to the type of lining-paper surface of an oviposition container (**a**) effect of six types of lining-papers functioned as an oviposition surface (see Table 1) (**b**) mean comparison using Fisher's Test. Here, A (plain printing offset paper 80 GSM), B (qualitative filter paper), C (agri seed germination paper KK030), D (agri seed germination paper KK029/85), E (agri seed germination paper-125), F (agri seed germination paper-75).

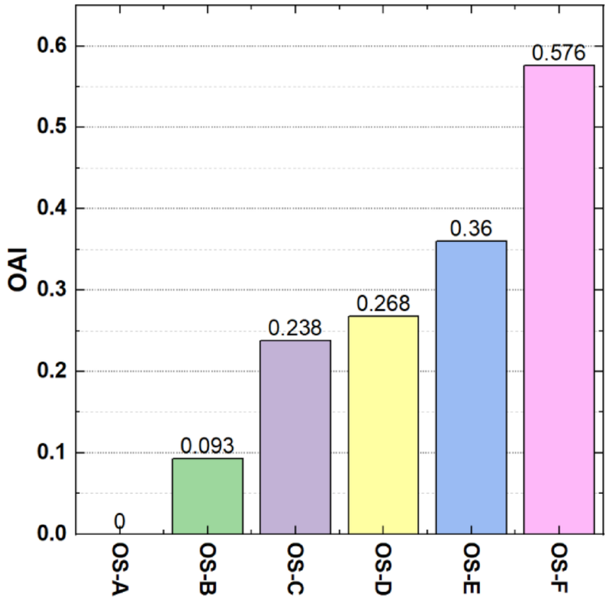

Fig. 8. Oviposition activity index (OAI) for a multi-choice test of *Aedes aegypti* gravid mosquitoes corresponding to different types of lining-papers (see Table 1) functioned as oviposition surfaces surrounding inner surface of an oviposition cup. Here, OS-A (plain printing offset paper 80 GSM), OS-B (qualitative filter paper), OS-C (agri seed germination paper KK030), OS-D (agri seed germination paper KK029/85), OS-E (agri seed germination paper-125), OS-F (agri seed germination paper-75).

In the case of *plain printing offset paper 80 GSM (OS-A)*, the value of the Oviposition Activity Index (OAI) was zero. For the rest of the five different oviposition lining-papers, the values of the Oviposition activity index (OAI) appeared between 0 and 0.576 (see Fig. 8).

Oviposition preference for different types of infusions

In the second experiment, the oviposition preference of *Ae. aegypti* gravid mosquitoes for different infusions was investigated. In this case, the *plain printing offset paper 80 GSM (OS-A)* was used as lining-paper which functioned as an oviposition surface attached to the inner surface of oviposition cups (see Table 2).

Overall, the mean number of eggs laid in oviposition cups by the gravid mosquitoes varied depending on the types of infusions added in the infusion cups (see Fig. 9a). Statistical analysis showed a statistically highly significant ($F = 8.629$; $df = 5, 12$; $P = 0.001$) correlation of oviposition preference with different water compositions (infusions) used in the oviposition cups. The mean comparison in pairs using Fisher's Least Significant Difference (LSD) test (see Fig. 9b) showed that the infusion containing *larval rearing water with Ae. aegypti larvae (IN-F)* were significantly different when compared with the *saline water (IN-A)*, *normal tap water (IN-B)*, or *only larval diet in water (IN-C)*. In the case of *larval rearing water with Ae. albopictus larvae (IN-E)*, the pairwise Fisher's LSD mean comparison was significantly different from *saline water (IN-A)* and *normal tap water (IN-B)* (see Fig. 9b). According to Fisher's LSD mean comparison test, no statistically significant effect (see Fig. 9b) was observed between the *larval rearing water with Ae. albopictus larvae (IN-E)* and *larval rearing water with Aedes aegypti larvae (IN-F)*.

In this case, the *normal tap water (IN-B)* for laboratory colony maintenance was considered as a control, where the value of the Oviposition Activity Index (OAI) was zero. For the rest of the five different infusions used in the oviposition cups, the values of the Oviposition activity index (OAI) appeared between − 1 and 0.5754 (see Fig. 10).

The highest oviposition activity was seen in the case of conspecific larval rearing water with their second instar larval stage *(IN-F)* used in the oviposition cup (see Fig. 10). All types of infusions in the oviposition cups functioned as attractant except the *saline water (IN-A)*. The application of *saline water (IN-A)* infusion has functioned negatively to oviposition preference.

Discussion

Effect of different oviposition surfaces

Aedes aegypti gravid mosquitoes prefer to lay their eggs on rough and moist surfaces of the oviposition sites[24,36–38]. It was reported that *Aedes* spp. expresses their preference to an oviposition surface with a moisture level similar to 70% of the soil SMC (saturation moisture content) to ensure the embryogenesis of eggs[39]. As different types of lining-papers have different water retention capacities as well as wet strengths, it was anticipated that the various types of lining-papers would have an impact on the oviposition preference. Previous research also indicated that female preference for ovitraps varies depending on the type of oviposition surface[29]. In their experiments, researchers also used different paper types (filter paper, blotting paper, paper towel, seed

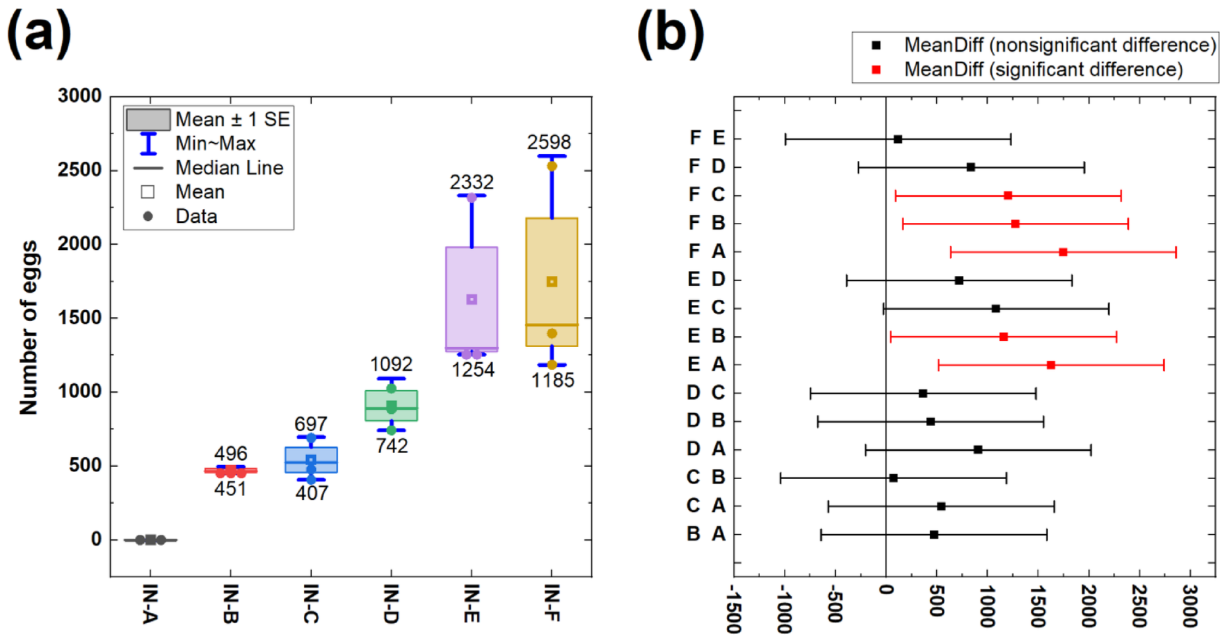

Fig. 9. Oviposition preference of *Aedes aegypti* gravid mosquitoes corresponding to different infusions (**a**) effect of six types of infusions in oviposition cups (see Table 2) (**b**) mean comparison using Fisher's Test. Here, A (saline water), B (normal tap water), C (only larval diet in water), D (*Aedes aegypti* larval-rearing water), E (larval-rearing water with *Aedes albopictus* larvae), F (larval rearing water with *Aedes aegypti* larvae).

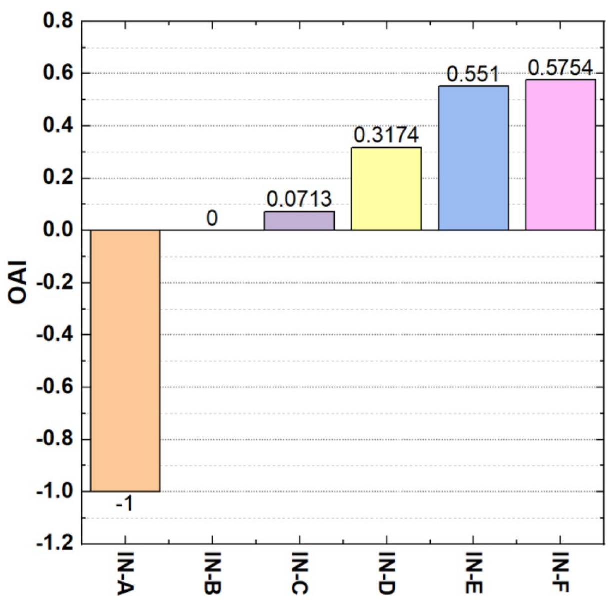

Fig. 10. Oviposition activity index (OAI) for a multi-choice test of *Aedes aegypti* gravid mosquitoes corresponding to different infusions (see Table 2) used in the oviposition cups located inside a bioassay cage. Here, IN-A (saline water), IN-B (normal tap water), IN-C (only larval diet in water), IN-D (*Aedes aegypti* larval-rearing water), IN-E (larval-rearing water with *Aedes albopictus* larvae), IN-F (larval rearing water with *Aedes aegypti* larvae).

germination paper) as oviposition substrates for *Aedes aegypti*[24,27–29,40–42]. However, systematic comparative study of oviposition preferences among various paper types remains unexplored. This study aims to evaluate the oviposition preferences of *Aedes aegypti* mosquitoes when presented with a selection of paper substrates that vary in properties.

In our experiment, we used different types of lining-papers that served as oviposition surfaces. The lining-papers attached to the inner surface of the oviposition cups/containers mimic the natural surface in laboratory conditions. As different types of lining-papers have different water retention capacities as well as wet strengths, it was anticipated that the various types of lining-papers would have an impact on the oviposition preference. Our results also showed that depending on the types of lining-papers, *Ae. aegypti* gravid mosquitoes showed statistically significant different oviposition preferences. In addition, our results also showed that seed germination papers of different variants (see Figs. 7 and 8) are more suitable as an oviposition surface due to higher oviposition activity in comparison with the *plain printing offset paper 80 GSM (OS-A)* or *qualitative filter paper (OS-B)*. This oviposition preference observed for seed germination papers appeared possibly due to the comparatively rougher surface and creped texture of these papers. The average surface roughness of the four selected seed germination paper types ranged from 43.46 to 97.90 µm (see Table 1), while the two plain paper types exhibited a comparatively lower average surface roughness, ranging from 2.91 µm to 9.08 µm. A study by O'Gower also reported a similar preference of *Aedes aegypti* for rough oviposition surfaces over smooth ones[38]. Moreover, the highest number of eggs were oviposited on *agri seed germination paper-75 (OS-F)*, possibly attributable to its relatively darker coloration in comparison to the other paper types examined (see Table 1 and Fig. 4). Similar brown colored paper towels were also used in several previous research studies[29,42]. The higher oviposition activity index could be attributed to the higher water retention capacity and surface roughness of the different variants of seed germination papers. The highest oviposition activity was observed in the case of *agri seed germination paper-75 (OS-F)*. Further research work is required to explore the exact reason for higher OAI in the case of *OS-F* paper.

Effect of different infusions

As the availability of food is essential for the survival of larvae, female mosquitoes were expected to choose oviposition sites containing rich organic matter to ensure food for their offspring[43,44]. In contrast to the findings of Brouazin et al.[24], our results evidently demonstrated that gravid mosquitoes prefer water containing a larval meal over normal tape water (see Figs. 9 and 10). This finding can be explained by the fact that the water in which mosquito larvae and pupae developed previously contained larval-produced excretory products and microbiota-derived metabolites that provided oviposition cues to attract gravid mosquitoes[24,45]. Those cues simulated naturally occurring oviposition pheromones specifically to help gravid mosquitoes in recognizing suitable oviposition sites for the development of newly hatched larvae[43,46,47]. Therefore, the presence of conspecific larvae in the infusions can be an indicator of a superior oviposition site. Likewise, the microbiota of larval rearing water also plays a vital role in generating an odorant that attracts gravid mosquitoes to the oviposition site[30,48]. For example, previous research revealed that *Aedes togoi* and *Ae. atropalpus* female mosquitoes show oviposition preference for the water (infusion) containing conspecific larvae reared in a sterile condition or water (infusion) in which fourth instar larvae were kept for 48 h. Moreover, their oviposition preference also depends on the density of the larvae in order to ensure enough resources for preadult development[47,49].

Our investigation revealed that, *Ae. aegypti* gravid mosquitoes display a significantly positive oviposition preference to conspecific larval rearing water (*IN-E* and *IN-F*) over all the other infusions used in our experiment (see Figs. 9 and 10). This finding also supports the previous study carried out by Allan & Kline where they observed similar oviposition responses between conspecific larval water and normal water[50]. However, they used larval rearing water stored at frozen temperature (− 20 °C for up to 3 weeks) whereas in this experiment we used larval rearing water stored at the temperature of 27 ± 1 °C for 24 h before the experiment. The findings of our experiment were also consistent with several previous research works involving larval water[24,47,51,52]. The oviposition preference pheromone associated with the presence of larval stage was elucidated by investigating behavioral and antennal responses of *Ae. aegypti* female mosquitoes to chemical cues from conspecific larvae[32,53,54]. For example, oviposition pheromone for *Ae. aegypti* and *Ae. albopictus* n-heneicosane (C21) was detected from the cuticle of their larval stage[54]. Carboxylic acid compounds such as 9-Octadecenoic acid (Z)-, methyl ester, dodecanoic acid, and tetradecanoic acid isomers extracted from larva were also observed to play a key role in the localization of oviposition sites by *Ae. aegypti* gravid mosquitoes[55].

In addition, salinity detection of the oviposition sites is also very important for the survival of *Ae. aegypti* larvae as contamination of water with seawater (as little as 12.5%) becomes lethal for their larvae[56]. In order to ensure larval survival, the oviposition preference of gravid mosquitoes decreases as salinity increases[46,57]. To detect lethal concentrations of saline water for their larvae, *Ae. aegypti* mosquitoes use specific neurons in their legs and mouthparts[14]. For this reason, in our experiment it was observed that the oviposition cup, containing simulated seawater (*IN-A)* with water salinity of about 35 ppt, repeals *Ae. aegypti* gravid mosquitoes from laying eggs (see Figs. 9 and 10). However, some *Aedes* species, for example *Ae. vigilax*, may prefer to oviposit in salt water[58].

It is evident from our study that larval rearing water with a conspecific larval stage (*IN-E* and *IN-F*) offers more cues to aid *Ae. aegypti* gravid mosquitoes in selecting their oviposition site compared to normal tap water (*IN-B*) or water containing only a larval diet (*IN-C*).

The findings from our oviposition preference study showed that both the types of lining-papers along with infusions used in oviposition cups have a significant effect on the oviposition preference for *Ae. aegypti* gravid mosquitoes. While preliminary, our study suggests that the *agri seed germination paper-75 (OS-F)* lining-paper in combination with the *larval rearing water with Ae. aegypti larvae (IN-F)* infusion could be used to design and develop a better oviposition cup that can be used in a bioassay cage or ovitrap[27,31,59–63].

Conclusion

In-depth understanding of the behavioral and breeding habits of *Aedes aegypti* is essential to control this vector by implementing an integrated pest management (IPM) approach where physical, biological, and chemical strategies can be used altogether. To study the behavioral and breeding habits of *Ae. aegypti,* a mimic of the natural breeding sites is usually built in a laboratory where highly efficient, species-specific container breeding mosquito population can be maintained as well as the effect of varying controlled environmental parameters can be observed[11]. In laboratory rearing of *Ae. aegypti* mosquitoes, oviposition preference of *Ae. aegypti* gravid mosquitoes varies depending on the types of lining materials that function as oviposition surfaces attached to the inner surface of oviposition cups or containers. In addition, the infusions used in oviposition cups show a significant effect on oviposition preference. Understanding *Ae. aegypti* mosquito breeding behavior which is influenced by different types of lining materials and infusions is essential to design and develop a better laboratory-rearing facility or ovitrap. This work presents a comparison study of the effect on the oviposition preference for six different types of lining-papers served as oviposition surfaces as well as six different types of infusions added in oviposition cups in a bioassay cage. It was observed that both the types of lining-papers along with infusions used in oviposition cups have a significant effect on the oviposition preference for *Ae. aegypti* gravid mosquitoes. Among the different types of lining-papers that functioned as oviposition surfaces, the highest oviposition preference was observed for the *agri seed germination paper-75 (OS-F)* in combination with the *normal tap water (IN-B)* infusion. Furthermore, six different types of infusions in the oviposition cups along with the *plain printing offset paper 80 GSM (OS-A)* lining-paper were used. In comparison with the *normal tap water (IN-B)* infusion, the *saline water (IN-A)* infusion showed a negative effect on the oviposition preference. The *larval rearing water with Aedes aegypti larvae (IN-F)* infusion showed the highest oviposition preference. Our study suggests that the *agri seed germination paper-75 (OS-F)* lining-paper in combination with the *larval rearing water with Aedes aegypti larvae (IN-F)* infusion could be used to design and develop a better oviposition cup that can be used in a bioassay cage or ovitrap. Overall, our observations indicate that different physical properties of lining-papers substantially influence oviposition behavior. However, our study did not take into account the chemical properties of the lining-papers. As chemical properties along with physical properties of lining-papers can also have an influence on oviposition preference, examining the role of chemical properties of paper and infusions along with physical properties can reveal new information that could be crucial for developing optimized substrates for mosquito bioassays and eco-friendly traps. Future research should systematically compare various paper treatments, including natural infusions, texture modifications, and color alterations, to identify the most attractive combinations for the targeted mosquito vector.

Data availability
The data in this study is available from the corresponding author on reasonable request.

Received: 24 September 2024; Accepted: 2 May 2025
Published online: 11 May 2025

References
1. McBride, C. S. et al. Evolution of mosquito preference for humans linked to an odorant receptor. *Nature* **515**, 222–227. https://doi.org/10.1038/nature13964 (2014).
2. Ponlawat, A. & Harrington, L. C. Blood feeding patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. *J. Med. Entomol.* **42**, 844–849. https://doi.org/10.1093/jmedent/42.5.844 (2005).
3. Akiner, M. M., Demirci, B., Babuadze, G., Robert, V. & Schaffner, F. Spread of the invasive mosquitoes *Aedes aegypti* and *Aedes albopictus* in the black sea region Increases risk of Chikungunya, Dengue, and Zika Outbreaks in Europe. *PLoS Negl. Trop. Dis.* **10**, 1–5. https://doi.org/10.1371/journal.pntd.0004664 (2016).
4. WHO. *Disease Outbreak News: Dengue—Global situation*, https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON498. Accessed 21 Dec 2023.
5. ECDC. *Chikungunya worldwide overview*, https://www.ecdc.europa.eu/en/chikungunya-monthly (2023).
6. Dalpadado, R., Amarasinghe, D., Gunathilaka, N. & Ariyarathna, N. Bionomic aspects of dengue vectors *Aedes aegypti* and *Aedes albopictus* at domestic settings in urban, suburban and rural areas in Gampaha District, Western Province of Sri Lanka. *Parasit. Vect.* **15**, 148–148. https://doi.org/10.1186/s13071-022-05261-3 (2022).
7. Valença, M. A., Marteis, L. S., Steffler, L. M., Silva, A. M. & Santos, R. L. C. Dynamics and characterization of *Aedes aegypti* (L.) (Diptera: Culicidae) Key Breeding Sites. *Neotrop. Entomol.* **42**, 311–316. https://doi.org/10.1007/s13744-013-0118-4 (2013).
8. Carvalho, F. D. & Moreira, L. A. Why is *Aedes aegypti* linnaeus so successful as a species?. *Neotrop. Entomol.* **46**, 243–255. https://doi.org/10.1007/s13744-017-0520-4 (2017).
9. Abeyewickreme, W., Gunathilaka, N., Ranathunge, T., Udayanga, L. & Wijegunawardena, A. Oviposition preferences of dengue vectors: *Aedes aegypti* and *Aedes albopictus* in Sri Lanka under laboratory settings. *Bull. Entomol. Res.* **108**, 442–450. https://doi.org/10.1017/S0007485317000955 (2018).
10. Crans, W. J. A classification system for mosquito life cycles: Life cycle types for mosquitoes of the northeastern United States. *J. Vect. Ecol.* **29**, 1–10 (2004).
11. Day, J. F. Mosquito oviposition behavior and vector control. *Insects* https://doi.org/10.3390/insects7040065 (2016).
12. Laursen, W. J. et al. Humidity sensors that alert mosquitoes to nearby hosts and egg-laying sites. *Neuron* **111**, 874-887.e878. https://doi.org/10.1016/j.neuron.2022.12.025 (2023).
13. Afify, A. & Galizia, C. G. Chemosensory cues for mosquito oviposition site selection. *J. Med. Entomol.* **52**, 120–130. https://doi.org/10.1093/jme/tju024 (2015).
14. Matthews, B. J., Younger, M. A. & Vosshall, L. B. The ion channel ppk301 controls freshwater egg-laying in the mosquito *Aedes aegypti*. *Elife* **8**, 1–27. https://doi.org/10.7554/eLife.43963 (2019).
15. Mayilsamy, M. Extremely long viability of *Aedes aegypti* (Diptera: Culicidae) eggs stored under normal room condition. *J. Med. Entomol.* **56**, 878–880. https://doi.org/10.1093/jme/tjy232 (2019).
16. Merritt, R. W., Dadd, R. H. & Walker, E. D. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annu. Rev. Entomol.* **37**, 349–376. https://doi.org/10.1146/annurev.en.37.010192.002025 (1992).
17. Sousa, N. A., Rodrigues, J., Luz, C. & Humber, R. A. Exposure of newly deposited *Aedes aegypti* eggs to *Metarhizium humberi* and fungal development on the eggs. *J. Invertebr. Pathol.* **197**, 107898. https://doi.org/10.1016/j.jip.2023.107898 (2023).
18. Colton, Y. M., Chadee, D. D. & Severson, D. W. Natural skip oviposition of the mosquito *Aedes aegypti* indicated by codominant genetic markers. *Med. Vet. Entomol.* **17**, 195–204. https://doi.org/10.1046/j.1365-2915.2003.00424.x (2003).
19. Mwingira, V., Mboera, L. E. G., Dicke, M. & Takken, W. Exploiting the chemical ecology of mosquito oviposition behavior in mosquito surveillance and control: a review. *J. Vector Ecol.* **45**, 155–179. https://doi.org/10.1111/jvec.12387 (2020).
20. James, L. D. et al. Field trials reveal the complexities of deploying and evaluating the impacts of yeast-baited ovitraps on *Aedes* mosquito densities in Trinidad, West Indies. *Sci. Rep.* **12**, 1–13. https://doi.org/10.1038/s41598-022-07910-0 (2022).

21. Montini, P. & Fischer, S. Oviposition site selection and subsequent offspring performance of *Aedes aegypti* in short- and long-term detritus accumulation conditions. *Acta Trop.* https://doi.org/10.1016/j.actatropica.2024.107222 (2024).
22. Farnesi, L. C., Menna-Barreto, R. F. S., Martins, A. J., Valle, D. & Rezende, G. L. Physical features and chitin content of eggs from the mosquito vectors *Aedes aegypti*, *Anopheles aquasalis* and *Culex quinquefasciatus*: Connection with distinct levels of resistance to desiccation. *J. Insect Physiol.* **83**, 43–52. https://doi.org/10.1016/j.jinsphys.2015.10.006 (2015).
23. Bar-Zeev, M. Oviposition of *Aedes aegypti* L. on a dry surface and hygroreceptors. *Nature* **213**, 737–738. https://doi.org/10.1038/213737b0 (1967).
24. Brouazin, R. et al. Optimization of oviposition trap settings to monitor populations of *Aedes* mosquitoes, vectors of arboviruses in La Reunion. *Sci. Rep.* **12**, 18450. https://doi.org/10.1038/s41598-022-23137-5 (2022).
25. Steinly, B. A., Novak, R. J. & Webb, D. W. A new method for monitoring mosquito oviposition in artificial and natural containers. *J. Am. Mosq. Control Assoc.* **7**, 649–650 (1991).
26. Chersoni, L. et al. The Possible role of microorganisms in mosquito mass rearing. *Insects* **12**, 645 (2021).
27. Allan, S. A. & Kline, D. L. Evaluation of organic infusions and synthetic compounds mediating oviposition in *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *J. Chem. Ecol.* **21**, 1847–1860. https://doi.org/10.1007/BF02033681 (1995).
28. Baak-Baak, C. M., Rodríguez-Ramírez, A. D., García-Rejón, J. E., Ríos-Delgado, S. & Torres-Estrada, J. L. Development and laboratory evaluation of chemically-based baited ovitrap for the monitoring of *Aedes aegypti*. *J. Vector Ecol.* **38**, 175–181. https://doi.org/10.1111/j.1948-7134.2013.12024.x (2013).
29. Musunzaji, P. S. et al. Oviposition preferences of *Aedes aegypti* in Msambweni, Kwale County, Kenya. *J. Am. Mosq. Control Assoc.* **39**, 85–95. https://doi.org/10.2987/22-7103 (2023).
30. Arbaoui, A. A. & Chua, T. H. Bacteria as a source of oviposition attractant for *Aedes aegypti* mosquitoes. *Trop. Biomed.* **31**, 134–142 (2014).
31. Obenauer, P. J., Kaufman, P. E., Allan, S. A. & Kline, D. L. Infusion-baited ovitraps to survey ovipositional height preferences of container-inhabiting mosquitoes in two Florida habitats. *J. Med. Entomol.* **46**, 1507–1513. https://doi.org/10.1603/033.046.0639 (2009).
32. Boullis, A. et al. Behavioural and antennal responses of *Aedes aegypti* (l.) (Diptera: Culicidae) gravid females to chemical cues from conspecific larvae. *PLoS ONE* **16**, e0247657–e0247657. https://doi.org/10.1371/journal.pone.0247657 (2021).
33. Tilak, R., Gupta, V., Suryam, V., Yadav, J. D. & Gupta, K. K. D. A laboratory investigation into oviposition responses of *Aedes aegypti* to some common household substances and water from conspecific larvae. *Med. J. Armed Forces India* **61**, 227–229. https://doi.org/10.1016/S0377-1237(05)80159-5 (2005).
34. Mourya, D. T., Gokhale, M. D., Barde, P. V. & Padbidri, V. S. A simple artificial membrane-feeding method for mosquitoes. *Trans. R. Soc. Trop. Med. Hyg.* **94**, 460–460. https://doi.org/10.1016/S0035-9203(00)90141-X (2000).
35. Kramer, W. L. & Mulla, M. S. Oviposition attractants and repellents of mosquitoes: oviposition responses of *Culex* mosquitoes to organic infusions. *Environ. Entomol.* **8**, 1111–1117. https://doi.org/10.1093/ee/8.6.1111 (1979).
36. Friuli, M. et al. Proof of concept of biopolymer based hydrogels as biomimetic oviposition substrate to develop tiger mosquitoes (*Aedes albopictus*) cost-effective lure and kill ovitraps. *Bioengineering* **9**, 267–267. https://doi.org/10.3390/bioengineering9070267 (2022).
37. Madeira, N. G., Macharelli, C. A. & Carvalho, L. R. Variation of the oviposition preferences of *Aedes aegypti* in function of substratum and humidity. *Mem. Inst. Oswaldo Cruz* **97**, 415–420. https://doi.org/10.1590/S0074-02762002000300025 (2002).
38. O'Gower, A. K. The influence of the surface on opposition by *Aedes aegypti* (Linn.)(Diptera, Culicidae). *Proc. Linnean Soc. New South Wales* **82**, 240–244 (1957).
39. Knight, K. L. & Baker, T. E. The role of the substrate moisture content in the selection of oviposition sites by *Aedes taeniorhynchus* (Wied.) and *A. sollicitans* (Walk.). [Research reports]. *Naval Med. Field Res. Lab. (Camp Lejeune)* **12**, 247–254 (1962).
40. Panigrahi, S. K., Barik, T. K., Mohanty, S. & Tripathy, N. K. Laboratory evaluation of oviposition behavior of field collected *Aedes* mosquitoes. *J. Insect.* **2014**, 207489. https://doi.org/10.1155/2014/207489 (2014).
41. Hernandez, E., Salim, K. & Joyce, A. Temperature influence on *Aedes aegypti* oviposition in the San Joaquin valley of California. *J. Am. Mosq. Control Assoc.* **39**, 216–222. https://doi.org/10.2987/23-7138 (2023).
42. Wong, J., Astete, H., Morrison, A. C. & Scott, T. W. Sampling considerations for designing *Aedes aegypti* (Diptera: Culicidae) oviposition studies in Iquitos, Peru: Substrate preference, diurnal periodicity, and gonotrophic cycle length. *J. Med. Entomol.* **48**, 45–52. https://doi.org/10.1603/me10149 (2014).
43. Albeny-Simões, D. et al. Attracted to the enemy: *Aedes aegypti* prefers oviposition sites with predator-killed conspecifics. *Oecologia* **175**, 481–492. https://doi.org/10.1007/s00442-014-2910-1 (2014).
44. Kroth, N. et al. Oviposition preferences of the mosquito *Aedes aegypti* Linnaeus, 1762 (Culicidae): An urban environment bioassay. *Bull. Entomol. Res.* **109**, 762–770. https://doi.org/10.1017/S000748531900021X (2019).
45. Mosquera, K. D. et al. Odor-mediated response of gravid *Aedes aegypti* to mosquito-associated symbiotic bacteria. *Acta Trop.* **237**, 106730. https://doi.org/10.1016/j.actatropica.2022.106730 (2023).
46. Gunathilaka, N., Ranathunge, T., Udayanga, L., Wijegunawardena, A. & Abeyewickreme, W. Oviposition preferences of dengue vectors; *Aedes aegypti* and *Aedes albopictus* in Sri Lanka under laboratory settings. *Bull. Entomol. Res.* **108**, 442–450. https://doi.org/10.1017/S0007485317000955 (2018).
47. Kalpage, K. S. P. & Brust, R. A. Oviposition attractant produced by immature *Aedes atropalpus*. *Environ. Entomol.* **2**, 729–730. https://doi.org/10.1093/ee/2.5.729 (1973).
48. Melo, N. et al. Geosmin attracts *Aedes aegypti* mosquitoes to oviposition sites. *Curr. Biol.* **30**, 127-134.e125. https://doi.org/10.1016/j.cub.2019.11.002 (2020).
49. Trimble, R. M. & Wellington, W. G. Oviposition stimulant associated with fourth-instar larvae of *Aedes togoi* (Diptera: Culicidae). *J. Med. Entomol.* **17**, 509–514. https://doi.org/10.1093/jmedent/17.6.509 (1980).
50. Allan, S. A. & Kline, D. L. Larval Rearing water and preexisting eggs influence oviposition by *Aedes aegypti* and *Ae. albopictus* (Diptera: Culicidae). *J. Med. Entomol.* **35**, 943–947. https://doi.org/10.1093/jmedent/35.6.943 (1998).
51. Serpa, L. L. N., Monteiro, S. D. C. B. & Voltolini, J. C. Efeito da água de criação larval na oviposição de *Aedes aegypti* em laboratório. *Rev. Soc. Bras. Med. Trop.* **41**, 515–517. https://doi.org/10.1590/S0037-86822008000500017 (2008).
52. Wong, J., Stoddard, S. T., Astete, H., Morrison, A. C. & Scott, T. W. Oviposition Site selection by the dengue vector *Aedes aegypti* and Its implications for dengue control. *PLoS Negl. Trop. Dis.* **5**, e1015. https://doi.org/10.1371/journal.pntd.0001015 (2011).
53. Faierstein, G. B., Lu, W., Sena, A. K. L. S., Barbosa, R. M. R. & Leal, W. S. Conspecific and allospecific larval extracts entice mosquitoes to lay eggs and may be used in attract-and-kill control strategy. *Sci. Rep.* **9**, 13747–13747. https://doi.org/10.1038/s41598-019-50274-1 (2019).
54. Gonzalez, P. V., González Audino, P. A. & Masuh, H. M. Electrophysiological and behavioural response of *Aedes albopictus* to n-heinecosane, an ovipositional pheromone of *Aedes aegypti*. *Entomol. Exp. Appl.* **151**, 191–197. https://doi.org/10.1111/eea.12184 (2014).
55. Wang, F. et al. Cartography of odor chemicals in the dengue vector mosquito (*Aedes aegypti* L., Diptera/Culicidae). *Sci. Rep.* https://doi.org/10.1038/s41598-019-44851-7 (2019).
56. Ponnusamy, L. et al. Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. *Proc. Natl. Acad. Sci.* **105**, 9262–9267. https://doi.org/10.1073/pnas.0802505105 (2008).

57. Clark, T. M., Flis, B. J. & Remold, S. K. Differences in the effects of salinity on larval growth and developmental programs of a freshwater and a euryhaline mosquito species (Insecta: Diptera, Culicidae). *J. Exp. Biol.* **207**, 2289–2295. https://doi.org/10.1242/jeb.01018 (2004).
58. Knight, J., Griffin, L., Dale, P. & Phinn, S. Oviposition and larval habitat preferences of the saltwater mosquito, *Aedes vigilax*, in a subtropical mangrove forest in Queensland, Australia. *J. Insect Sci.* https://doi.org/10.1673/031.012.0601 (2012).
59. Leite Alves, A. C. et al. Attractive activity of plant extracts for the oviposition of *Aedes aegypt*i L. (Diptera: Culicidae). *Idesia (Arica)* https://doi.org/10.4067/S0718-34292018005000502 (2018).
60. Mackay, A. J., Amador, M. & Barrera, R. An improved autocidal gravid ovitrap for the control and surveillance of *Aedes aegypti*. *Parasit. Vect.* **6**, 225–225. https://doi.org/10.1186/1756-3305-6-225 (2013).
61. Parker, C. Collection and rearing of container mosquitoes and a 24-h addition to the CDC bottle bioassay. *J. Insect Sci.* https://doi.org/10.1093/jisesa/ieaa059 (2020).
62. Ritchie, S. A., Long, S., Hart, A., Webb, C. E. & Russell, R. C. An adulticidal sticky ovitrap for sampling container-breeding mosquitoes. *J. Am. Mosq. Control Assoc.* **19**, 235–242 (2003).
63. Shu, C.-Y. & Shelomi, M. *Syzygium samarangense* leaf infusion as *Aedes albopictus* (Diptera: Culicidae) ovitrap bait. *J. Med. Entomol.* **58**, 965–968. https://doi.org/10.1093/jme/tjaa231 (2021).

Author contributions

Mahfuza Momen: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing original draft, Writing review & editing. Kajla Sheheli: Funding acquisition, Writing review & editing. Md. Aftab Hossain: Writing review & editing. Ananna Ghosh: Investigation, Writing-review & editing. Md. Forhad Hossain: Writing-review & editing. All authors reviewed the manuscript.

Funding

This work was supported by Bangladesh Atomic Energy Commission (BAEC) and International Atomic Energy Agency (IAEA) under IAEA Coordinated Research Project D44004: Mosquito Irradiation, Sterilization and Quality Control (2020–2025).

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to M.M.

Reprints and permissions information is available at www.nature.com/reprints.