

ORIGINAL ARTICLE

**DEVELOPMENT OF A DISSEMINATION PROTOTYPE
DEVICE FOR FOCAL AEDINE MOSQUITO CONTROL**

*Juscelino Rodrigues, Juan Mercado Martinez and Christian Luz***ABSTRACT**

Focal control techniques are among the vital tools for integrated control of aedine vectors of arboviruses to humans in the tropics and subtropics. This study sought the development of a simple dissemination device to attract *Aedes aegypti* adults and the validation of this device in laboratory and field conditions. The attractiveness of aqueous and dry substrates, in simple containers or in the prototype of a dissemination device for gravid *A. aegypti* to oviposit was assessed in two- or multi-choice tests in the laboratory or under field conditions in the city of Goiânia in Central Brazil. Findings emphasized the utility of a hydrogel (polyacrylamide potassium polyacrylate) as a substitute for liquid water in the device but did not confirm that any of the tested substances, acetone, lactic acid, orange oil, and bamboo leaf infusion, incorporated in the gel, clearly attracted gravid females to oviposit. The plastic dissemination device (total 29.5 cm height) was divided into a lower closed compartment with a water reservoir and an upper capped compartment permitting free circulation of mosquito adults. This compartment contained hydrated gel in the base, and black polyethylene terephthalate carpet covered the lateral inner surface. The device attracted *A. aegypti* gravid females seeking a breeding site, and females laid eggs on the gel and the carpet. Findings in laboratory and field conditions strengthen the value and utility of this device for focal applications of insecticides against this important mosquito vector.

KEY WORDS: Mosquito; *Aedes aegypti*; oviposition attractant; substrate.

INTRODUCTION

Increasing perception about the complexity of mosquito vector control urges efforts to develop and improve multisided tools for integrated control of specific key vectors. Widespread applications of synthetic insecticides proved inopportune routine strategies against synanthropic mosquito vectors due to their detrimental impact on non-target organisms and the risk of generating insecticide-resistant vector populations (Smith et al., 2016). Innovative techniques counting on biological formulations and strategies linked to a sound understanding and

Universidade Federal de Goiás, Instituto de Patologia Tropical e Saúde Pública, Goiânia, Goiás, Brazil

ORCIDs

Juscelino Rodrigues: <https://orcid.org/0000-0002-1277-8966>

Juan Mercado Martinez: <https://orcid.org/0000-0001-7469-4920>

Christian Luz: <https://orcid.org/0000-0002-5231-0735>

Corresponding author: Christian Luz. E-mail: wolf@ufg.br

Received for publication: 4/7/2024. Reviewed: 21/10/2024. Accepted: 29/10/2024.

implementation of behavioral patterns of the target vector open encouraging prospects to contain mosquito-borne diseases in humans (Roiz et al., 2018).

Focal mosquito control is applied by different means in restricted, sensitive locations. So-called traps intend to attract and retain mosquitoes after contact by mechanical, chemical, or physical impacts and to cause immediate or delayed death of trapped individuals due to starvation and dehydration (Reiter et al., 1991; Donatti & Gomes, 2007; Barrera et al., 2013; Kumawat et al., 2014). Dissemination devices, in contrast, enable a free and continuous circulation of mosquitoes in the device and its surroundings (Wang et al., 2014; Buckner et al., 2017). Mosquitoes get contaminated with active agents (e.g., an entomopathogen or synthetic insecticide) when entering the device and, upon leaving, disperse those agents to other habitats such as cryptic breeding and resting sites and to other adults. Entomopathogenic fungi infect mosquitoes, and vector competence of diseased females is affected, and adults eventually succumb to infection. There are intense studies to develop new, more effective, cost-efficient, long-lasting, and manageable trap and dissemination devices. A multitude of low-to-high-cost commercialized traps are available in many countries, and home-made trap devices are popularized in social media. Efforts to develop dissemination devices for mosquito control are just beginning.

A crucial point in focal control with trap or dissemination devices is to guarantee acceptance and attractiveness of devices to the target mosquito adults and their competitiveness with other resting and breeding sites available in intra- and peridomestic areas where the vector occurs and that cannot be eliminated. Locomotion of diurnal aedine adults towards a device simulating a breeding or resting site is triggered by visual, olfactory, tactile, and abiotic stimuli and depends on the mosquito species, gender, and physiological age. *Aedes aegypti* is a key vector of arboviruses that infect humans in the tropics and subtropics (Weaver et al., 2018; Brady & Hay, 2020). Females of this and other aedine species are anthropophilic and commonly lay their eggs above the water line in small- to middle-sized, human-made or natural, transient, or permanent standing water volumes with low organic material loads where immature stages develop. When not actively flying or otherwise moving, diurnal male and female adults move to hiding and resting places in protected indoor and outdoor sites that are often darker and more humid than the surrounding area.

We report here on laboratory and field choice tests of a simple prototype dissemination device for focal control of *A. aegypti* and other synanthropic aedine mosquitoes.

MATERIAL AND METHODS

Origin, rearing of Aedes aegypti, and preparation of adults

The colony of *A. aegypti* originated from larvae collected in the city of Goiânia, Brazil, in 2012 and was maintained in the laboratory following Rocha et al. (2015). The females were fed as described by Lima et al. (2009),

a method approved by the Ethics Commission for the Use of Animals, UFG, Goiânia, CEUA 032/18, UFG, May 28, 2018. Before each test, enough adult females and males were aged to 5-6 days and continuously offered a 10% sucrose solution while females were blood-fed twice (Martinez et al., 2021).

Preparation of substrates and additives

Aqueous and solid substrates were used to test the oviposition behavior of gravid females in two- or multi-choice tests. The aqueous substrates were sterile distilled water and hydrogel, a network of hydrophilic copolymer chains. Sterile distilled water was generally tested as a control without any additives. Granulated hydrogel (polyacrylamide potassium polyacrylate; Forth Gel®; Forth Jardim, Tietê, Brazil), referred to hereafter as gel, was agitated with a magnetic stirrer (Fisatom® 752A, Fisatom, Equipamentos Científicos; São Paulo, Brazil) with sterile distilled water at 0.6% of the gel. The gel was tested without additives or with added acetone (Isofar PA, Ref. 1210, Duque de Caxias, Brazil), lactic acid (Isofar PA, Ref. 116), or orange oil (Kreidezeit, Ref. 450; Sehlem, Germany) at concentrations of 0.001%, 0.005%, 0.01%, 0.05% and 0.1%. Another additive, an infusion of bamboo leaves, was tested at 0.1%, 1%, 10%, 50% and 100%. The infusion was prepared with senescent dry leaves of bamboo (*Bambusa vulgaris vittata*, Poaceae) collected from a residential area in Terezópolis, Goiás, Brazil, and dried at 40 °C for 24 h. Leaf biomass (10 g/L infusion) was mixed with distilled water and kept in a closed container at room temperature for two weeks before the beginning of the experiment.

Containers (200 mL) with their inner glossy surface unaltered or roughened with sandpaper (A-257, G220, Norton Saint-Gobain Abrasivos; Guarulhos, Brazil) were filled with 125 mL of distilled water or gel with or without one of the additives noted above at specific concentration. In other tests, black polyethylene terephthalate (PET) carpet (Ecotex®, Inylbra, São Paulo, Brazil) with a layer thickness of 2 or 4 mm (with a fine- or coarse-grained surface, respectively) was tested as a solid substrate in a dissemination device arranged with gel plus additives or water only.

Devices

Two different types of test devices were used: a transparent colorless polypropylene container (200 mL; 8.5 cm diameter) tested only in laboratory conditions (Figure 1a), and a prototype dissemination device (Figure 1b, c) tested in laboratory and field conditions. The 200-mL containers were tested with different additives and different additive concentrations (multi-choice tests), and the dissemination device with or without orange oil at a single concentration (two-choice tests).

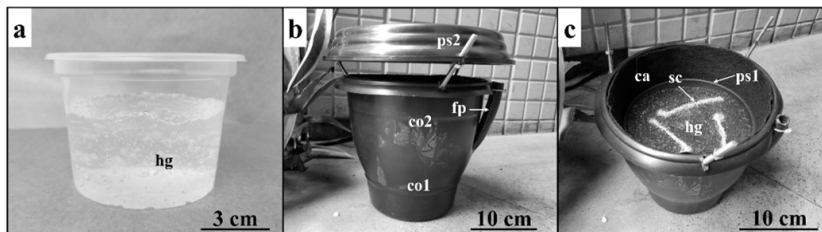


Figure 1. Plastic container (200 mL) with hydrogel [hg] (a) and dissemination device, covered (b) and oblique view into the uncovered inner upper space (c) consisting of a closed water reservoir at the base [co1] and upper open compartment [co2], both separated by a pot saucer filled with hydrogel [ps1], three synthetic cords [sc] connecting water through drilled holes in the bottom of the saucer with hydrogel, filler plug [fp], cover [ps2] and carpet [ca].

The dissemination device consisted of a black polyethylene circular container (21 cm height and upper 21.5 cm and 14 cm lower diameters; Figure 1b, c). A black polyethylene pot saucer (19 cm diameter and 3.5 cm height) set horizontally inside the device divided the container into a lower closed compartment with water as a reservoir and an upper open compartment. The pot saucer was filled with 500 mL gel, and the gel was hydrated by three synthetic fiber cords inserted in three holes drilled in the bottom of the saucer with overlapping cords sections into the water reservoir and the gel in the saucer. A lockable filler plug at the outside container connected through a tube with the reservoir permitted refilling the reservoir with water. Black PET carpet covered the inside of the device above the pot saucer. A second black polyethylene pot saucer (28 cm diameter and 4.5 cm height) was fixed upside down as a cover on the container with three stainless bolts (6 cm length and 0.5 cm diameter) at equal distance from each other to leave a free space of 4 cm between the container and cover to permit free circulation of mosquito adults (Martinez et al., 2021).

Laboratory tests

Laboratory two- or multi-choice tests were performed with containers or dissemination devices in screened cages (80 cm width, 80 cm height, and 60 cm length). Routinely, 30 female and 30 male adults were tested in each cage. Adults were perpetually offered two vials with 10% sucrose solution, and a filter paper wick, and females were blood-fed for 30 min twice a week (Rocha et al., 2015). Relative humidity (RH) and temperature inside the cage at a 40 cm height and in the dissemination device (when used) were recorded with a MX2304 data logger (Onset® MX2304; Onset Computer Corporation, Bourne, USA). Eggs laid on substrates or the inner surface of the container or on the carpet in the dissemination device were quantified and retrieved daily

for 10 days. Cages were checked daily for dead adults. After each assay, cages were carefully cleaned with a cloth soaked with 70% ethanol, and all surfaces were sprayed with a 3% hypochlorite solution. This procedure was repeated 24 hours before using the cage in a new test.

Field tests on the device performance

Field tests with dissemination devices were done between February and July (transitioning between the late rainy into the dry seasons) 2019 in metropolitan Goiânia, Central Brazil. In 2019, in each of four residences with permanent presence of *A. aegypti* in peridomestic areas, two dissemination devices were set at least 1 m apart on the ground protected from direct solar radiation and rains. In one device, gel with 0.001% orange oil and in the other, only gel was tested. Weekly, the carpet and gel with or without orange oil were retrieved, transferred to transparent plastic bags, and replaced. In the laboratory, all samples of gel and carpet were checked for eggs, and eggs were quantified with a stereomicroscope and a manual cell counter (Digitimer® Mod. 16103; Digitimer, Hertfordshire, England). A 10% sample of the egg batches in each device was kept in a humid chamber and then submerged in water; eclosing larvae were processed as mentioned until the emergence of adults (Rocha et al., 2015) and then identified morphologically (Rueda, 2004). Precipitation rates in Goiânia during field tests in 2019 were obtained from the Meteorological Station Goiânia A002, OMM 86734 (<http://www.inmet.gov.br/portal/> accessed on 27 August 2019).

Statistical Analyses

Unless otherwise mentioned, each test was performed with four independent repetitions with different substrates and additives. Data was analyzed with analysis of variance (*F*) or non-parametric Kruskal Wallis test (*H*) and the Student-Newman-Keuls (SNK) multiple test of comparison (Statistica 7.1; StatSoft, Tulsa, USA). Means were significantly different at $p < 0.05$.

RESULTS

Laboratory two-choice tests with substrates in containers

Within one and three days of exposure in cages, females started to lay eggs in containers filled with water or gel, respectively. In containers tested with water, > 90% of eggs were laid on the plastic above the waterline. In containers tested with gel, 100% of the eggs were laid on the surface of the gel. Egg numbers subsequently increased to a total of 28 ± 8.7 eggs/female in containers with water and 17 ± 3.3 eggs/female in containers with gel (total mean number of 44.4 ± 10.2 eggs/female in each cage) after a 10-day exposure

with no significant effect of the water or gel substrate on the number of eggs ($H_{1,8} = 1.3$; $p = 0.2$; Figure 2a). The mean mortality of adults in the cage in the same period was $1.3 \pm 0.9\%$, and only males had died.

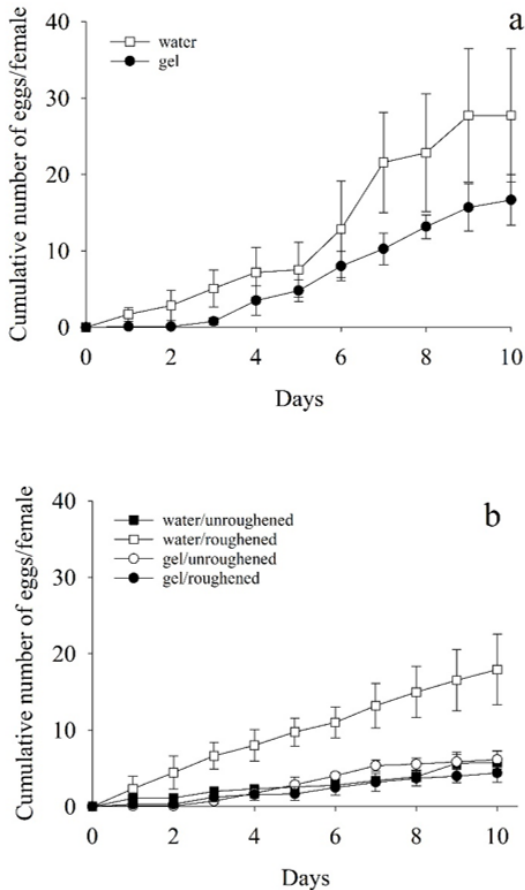


Figure 2. Cumulative mean number (\pm standard error) of eggs laid by *Aedes aegypti* in plastic containers (200 mL) with glossy inner surface provided with water or gel (two-choice tests; a) or in containers provided with water or gel and their inner surface roughened or not (multi-choice tests; b); tests were run in cages with 30 females and 30 males, each cage, at $25 \pm 2^\circ\text{C}$ and $75 \pm 10\%$ relative humidity during 10 days (cumulative mortality $< 5\%$ after a 10-day exposure regardless of the test).

Laboratory multi-choice tests with substrates and additives in containers

In multi-choice tests, caged females started to lay eggs within the first 24 h of exposure to containers, whether provided with water or gel (without additives), and whether the inner container surface was roughened or not. Most eggs (18 ± 4.6 eggs/female) were laid in the following 10 days in containers with water and a roughened inner surface. The values of accumulated eggs in the other containers at the same period varied between 4.4 ± 1.2 eggs/female in containers with gel and roughened surface and 6.1 ± 1.2 eggs/female in containers with gel and unroughened inner surface (Figure 2b). The total mean number of eggs/females in this test was 34 ± 4.1 after a 10-day exposure. There was a significant effect of the preparation of the container (condition of the inner surface and aqueous substrate) on the number of eggs laid ($H_{3,16} = 8.1$; $p = 0.04$; water/roughened inner surface > all other combinations).

In further multi-choice tests in cages with containers and an inner glossy unroughened surface prepared with water, gel, and gel plus added acetone, lactic acid, bamboo leaf infusion, or orange oil at different concentrations, females started to lay eggs on the plastic at the waterline (90%; and the rest of the eggs floated on the water surface or settled to the bottom of the container) or randomly on the gel surface (100%) within 24 h (Figure 3a-d). In the next nine days, most eggs were laid on gel prepared with leaf infusion (total 33 ± 4.3 eggs/female), followed by acetone (total 25.6 ± 5.5 eggs/female), orange oil (total 20.2 ± 4.6 eggs/female) and lactic acid (total 18.3 ± 4.3 eggs/female) with no significant effect of the additive (infusion, orange oil, acetone or lactic acid) on the number of eggs ($H_{3,28} = 3.6$; $p = 0.3$). Females laid ≤ 3.5 eggs/female in containers with gel added with acetone regardless of the concentration, and most eggs were laid in control containers with water only (12.5 ± 3.9 eggs/female) (Figure 3a). However, there was no effect of the container preparation with acetone at different concentrations or water (control) on the total number of eggs/female/container ($H_{6,28} = 10.5$; $p = 0.1$). In addition, no significant effect of the container preparation on the total number of eggs/female/containers was detected for both lactic acid and infusion at the concentrations tested ($H_{6,28} \leq 12.5$; $p = 0.05$). The number of eggs/female/container varied here between 1.2 ± 0.7 (infusion at 50%) and 8.9 ± 2.5 eggs/female (control water testing infusion; Figure 3b, c). Testing orange oil, the number of eggs/females was significantly higher ($H_{6,28} = 14$; $p = 0.02$) in containers with water (7.2 ± 2.5 eggs), gel (3.4 ± 2.1 eggs), lowest 0.001% (4 ± 0.3 eggs) and highest 0.1% of the oil (3.3 ± 1.5 eggs) than the other concentrations tested ($\leq 1.1 \pm 0.7$ eggs; Figure 3d).

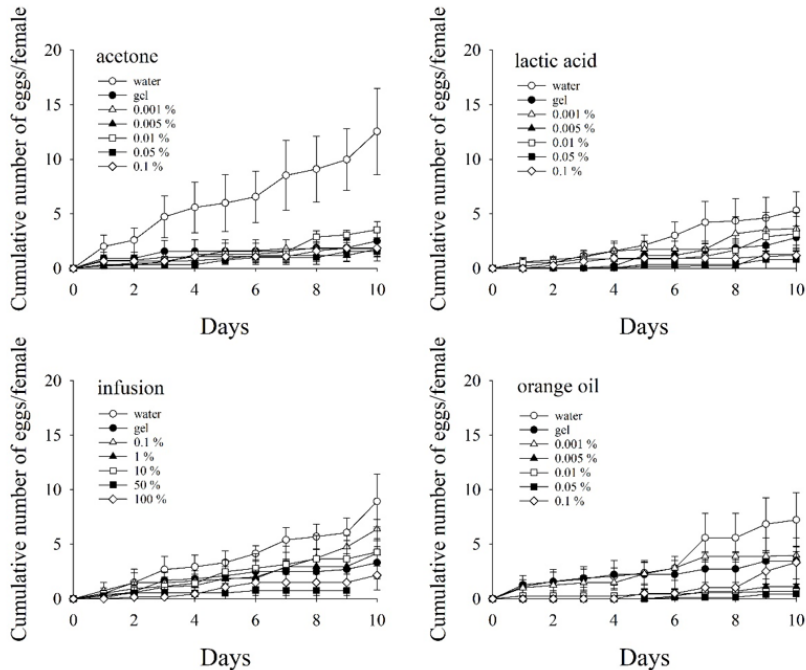


Figure 3. Cumulative mean number (\pm standard error) of eggs laid by *Aedes aegypti* in plastic containers (200 mL) with glossy inner surface provided with water, gel (controls) or gel added with acetone (a), lactic acid (b), bamboo leave infusion (c) or orange oil (d) at different concentrations (multi-choice tests for each substance at different concentrations); tests were run in cages with 30 females and 30 males in each cage, at 25 ± 2 °C and $75 \pm 10\%$ relative humidity during 10 days (cumulative mortality $< 5\%$ after a 10-day incubation, regardless of the test).

Laboratory two-choice tests with dissemination devices

Females exposed in cages with two disseminating devices set up with gel and carpet of different textures and layer thickness entered devices and started to lay eggs on the gel and either coarse- or fine-grained carpet within 24 h. The total number of eggs after a 10-day exposure of females to devices with coarse- and fine-grained carpet reached 17.5 ± 5.5 and 18.5 ± 3 eggs/female, respectively, with no significant effect of the texture of the carpet tested on the number of eggs ($H_{1,8} = 7$; $p = 0.3$; Figure 4). In both devices, the total number of eggs/female laid on the gel (16.7 ± 5.5 and 17.5 ± 3.2 eggs/female in devices set up with coarse- and fine-grained carpet, respectively) was significantly higher than the number of eggs laid on coarse-grained (0.3 ± 0.1 egg/female) or fine-grained carpet (1 ± 0.3 egg/female) ($H \leq 5.4$; $p = 0.02$; Figure 4).

Minimum and maximum temperature and relative humidity monitored daily in this test performed in the laboratory during part of June 2018 varied between 21 °C and 23.1 °C and between 95.1% and 100%, respectively, in the interior of the device and between 21.5 °C and 23.6 °C and between 64.7% and 82.6%, respectively, in the exterior of the device in the cage (Figure 1S, supplementary material).

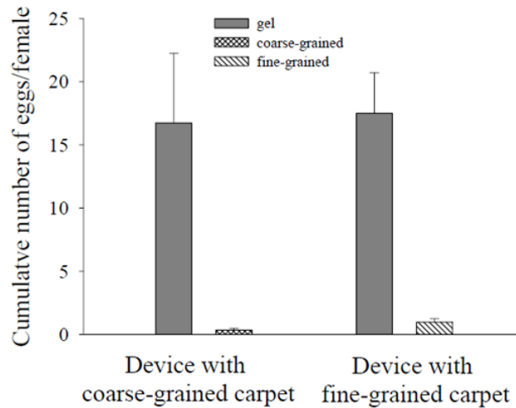


Figure 4. Cumulative mean number (\pm standard error) of eggs laid by *Aedes aegypti* on gel and carpet of different texture and layer thickness adjusted in a dissemination device (two-choice tests); tests were run in cages with 30 females and 30 males in each cage, at 25 ± 2 °C and $75 \pm 10\%$ relative humidity for 10 days (cumulative mortality < 5%).

Field two-choice tests with additive

The total number of eggs collected in dissemination devices arranged with gel mixed with orange oil or gel only and exposed in peridomestic areas of four sites in Goiânia between March 26 and July 26, 2019, varied between lowest 3,948 eggs (residence no 1) and highest 7,795 eggs (residence 4). There was no significant effect of the orange oil in the gel ($H_{1,144} = 1.8$; $p = 0.17$) or of the locale ($H_{3,72} = 7.3$; $p = 0.06$) on the number of eggs. In the first month, the cumulative weekly number of eggs was generally low and did not exceed 110, regardless of the locale. The number increased in the last week of April and remained substantial until the end of the test in July. The highest total weekly relative mean number of eggs was found in devices arranged with gel mixed with orange oil (290 ± 71.9 eggs; 07/06/2019; Figure 5), with 74.3% of the eggs laid on the gel and the remaining eggs on the carpet. The weekly mean number of eggs found in devices tested with gel mixed with orange oil varied between 215.7 ± 42.6 eggs (07/06/2019) and 18.3 ± 5.2 eggs (09/04/2019) on the gel and between 130.5 ± 59.9 eggs (10/05/2019) and 10 ± 3.5 eggs (09/04/2019) on the

carpet (Figure 5). In devices tested with gel and without the oil, the relative weekly mean number of eggs on the gel varied between 181 ± 26.5 eggs (31/05/2019) and $6.8 \pm 1.8\%$ eggs (09/04/2019) and on the carpet between 85.2 ± 31.1 eggs (23/04/2019) and 4.8 ± 1.6 eggs (09/04/2019; Figure 5). Significantly more eggs were laid on the gel, whether mixed with ($H_{1,44} = 6.7$; $p = 0.01$) or without orange oil ($H_{1,44} = 7.9$; $p = 0.05$) than on the carpet.

Daily minimum and maximum temperature, relative humidity, and the cumulative precipitation between February and July 2019 monitored by the Meteorological Station in Goiânia are presented in Figure 2S (supplementary material). Daily precipitation in the first 6 weeks did not exceed 27.2 mm, and in April, several significant rainfalls up to 66 mm were registered. The rains definitively stopped in Goiânia in the middle of May. In the same period, both temperature and relative humidity decreased from their highest levels of 26.9 °C and 87.3% RH in March to their lowest levels, 14.1 °C and 40% RH, in July (Figure 2S).

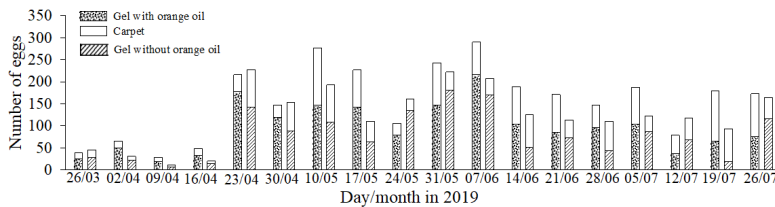


Figure 5. Weekly mean cumulative number of aedine eggs laid on carpet and gel, mixed with (0.001%) or without orange oil in a disseminating device exposed in four different peridomestic areas in Goiânia, Brazil, between March and July 2019.

DISCUSSION

The validity of the outcome of two- or multiple-choice tests, whether two or multiple employed here, is generally limited, especially in laboratory conditions, and results often diverge under field conditions due to the complexity of uncontrolled interacting factors. However, these tests are essential, and their findings frequently provide valuable clues to new concepts to test in field conditions. In simple containers filled with water, *A. aegypti* females clearly preferred to oviposit on dry plastic close to the waterline and generally not to oviposit directly on a water surface, a behavior pattern triggered by climatic conditions and previously reported for this mosquito (Madeira et al., 2002; Day, 2016). In fact, wettable and moist substrates strongly attract gravid *A. aegypti* females (Day, 2016). Gravid females here accepted and clearly preferred the aqueous gel substrate for oviposition over the dry glossy plastic of the container.

The gel proved to be a useful and cheap substrate that can easily be kept hydrated and mixed or superficially treated with synthetic or biological insecticides and attractants useful for extended periods in dissemination or trap devices for controlling this vector. The orientation of females towards a breeding site is also triggered by such volatile semiochemicals as acetone, lactic acid and limonene or mixtures of compounds in infusions produced by microbial decay of organic material in the breeding sites (Allan & Kline, 1995; Sant'Ana et al. 2006; Ponnusamy et al., 2008, Navarro-Silva et al., 2009; Arbaoui & Chua, 2014; Albeny-Simões et al., 2014; Ponnusamy et al., 2015).

None of the potential semi-chemicals tested here in the gel conclusively attracted gravid females to containers in the laboratory or to the disseminating device tested with orange oil at field conditions. Acetone and lactic acid emanate from human skin (Bernier et al., 1999, 2007) but also, together with limonene - a major fraction of orange oil - are products of microbial activity in organic infusions (Thorn et al., 2001; Sant'Ana et al., 2006; Arbaoui & Chua, 2014). Acetone, lactic acid, and leaf infusion in the present tests did not disrupt oviposition behavior, regardless of concentration. Increasing concentrations of orange oil repelled gravid females, although, surprisingly, the highest concentration (0.1%) of this oil did not repel gravid females. The performance of the leaf infusion, with highest numbers of eggs in the present study, and of other leaf infusions to attract gravid mosquitoes depends on the qualitative and quantitative composition of semiochemicals produced by the developing microbiota on the decaying organic material and by ambient temperature and time of fermentation (Navarro-Silva et al., 2009; Ponnusamy et al., 2008, 2015; Wong et al., 2011; Arbaoui & Chua, 2014).

Infusions, unlike the other substances tested, are, by their very nature, difficult to standardize but are promising, low-cost, and easily prepared resources. Offensive odors due to high concentrations of any infusions should be avoided. However, as was seen with orange oil, the concentration of a substance can induce indifference or even deterrence (by repulsion) to ovipositing females. In previous tests, limonene was attractive to anopheline adults (Nyasembe et al., 2012) and gravid aedine females (Sant'Ana et al., 2006), but that result was not clearly shown for the concentrations of orange oil mixed with the gel in laboratory conditions tested here. The results obtained with orange oil in the disseminating device in the field are encouraging but also inconclusive. Orange oil, an accessible and cheap resource with a pleasant odor, or infusions of leaves with undefined compounds and unpleasant odor, were the most promising options to attract gravid *A. aegypti*, but the utilities of both need more rigorous evaluation in field conditions.

Roughening of the inner plastic surface of containers and carpet, either coarse- or fine-grained, arranged in the dissemination device proved critical to attracting gravid females to oviposit. The carpet was well accepted by gravid females that showed no clear ovipositional preference for a specific texture of

the carpet tested. In laboratory tests, eggs were randomly laid on the carpet, which was not in direct contact with the gel. However, most eggs were found randomly scattered on the gel's surface. During field tests in the dry season in 2018 with devices charged with gel or water and set in peridomestic areas in Goiânia, *A. aegypti* laid significant numbers of eggs randomly on fine-grained carpet, regardless of the aqueous substrate (water or gel), while the gel occasionally seemed to increase attractiveness for oviposition (Martinez et al., 2021). Plastic materials such as carpet and gel that resist decay by an uncontrolled microbiota improve device standardization and increase the durability and effectiveness of replaceable segments that carry substrates treated with the insecticidal formulation. The choice of the carpet, whether fine- or coarse grained, will be determined by the suitability as a substrate for a specific insecticidal formulation. The dark, fine-grained carpet tested here was easier to handle and to fix in the device than the coarse-grained carpet. It proved to be very suitable as a substrate carrying a granular formulation of *Metarhizium humberi* propagules. Further use in the dissemination device proved effectiveness against *A. aegypti* in laboratory, semi-field and field tests (Rodrigues et al., 2021, Martinez et al., 2021).

The present prototype of a simple dissemination device provides valuable means particularly to use formulations with entomopathogenic fungi for focal control of *A. aegypti* or other synanthropic mosquito vectors that seek small-sized water bodies.

ACKNOWLEDGMENTS

The authors thank Lucas R. Oliveira for technical assistance and Richard A. Humber for the critical review of the manuscript. This study was supported by the National Council for Scientific and Technological Development, CNPq, Coordination of the Improvement of Higher Education, CAPES/MEC, CNPq/MCTI and Brazilian Ministry of Health, Decit/SCTIE/MS (440506/2016) and scholarships to JMM, JR (both CAPES). We also thank CNPq for the grant PQ 311672/2016-7 to CL.

CONFLICT OF INTEREST

The authors declare no conflict of interest or financial ties to disclose.

REFERENCES

1. Albeny-Simões D, Murrell EG, Elliot SL, Andrade MR, Lima E, Juliano AS, Vilela EF. Attracted to the enemy: *Aedes aegypti* prefers oviposition sites with predator-killed conspecifics. *Oecologia* 175: 481-492, 2014.

2. Allan S, Kline DL. Evaluation of organic infusions and synthetic compounds mediating oviposition in *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *J Chem Ecol* 21: 1847-1860, 1995.
3. Arbaoui AA, Chua TH. Bacteria as a source of oviposition attractant for *Aedes aegypti* mosquitoes. *Trop Biomed* 31: 134-142, 2014.
4. Barrera R, MacKay AJ, Amador M. A novel autocidal ovitrap for the surveillance and control of *Aedes aegypti*. *J Am Mosquito Contr* 29: 293-296, 2013.
5. Bernier UR, Booth MM, Yost RA. Analysis of human skin emanations by gas chromatography/mass spectrometry. I. Thermal desorption of attractants for the yellow fever mosquito (*Aedes aegypti*) from handled glass beads. *Anal Chem* 71: 1-7, 1999.
6. Bernier UR, Kline DL, Allan SA, Barnard DR. Laboratory comparison of *Aedes aegypti* attraction to human odors and to synthetic human odor compounds and blends. *J Am Mosqu Control Assoc* 23: 288-293, 2007.
7. Brady OJ, Hay SI. The global expansion of Dengue: How *Aedes aegypti* mosquitoes enabled the first pandemic arbovirus. *Annu Rev Entomol* 65: 191-208, 2020.
8. Buckner EA, Williams KF, Marsicano AL, Latham MD, Lesser CR. Evaluating the vector control potential of the In2care mosquito trap against *Aedes aegypti* and *Aedes albopictus* under semifield conditions in Manatee County, Florida. *J Am Mosqu Control Assoc* 33: 193-199, 2017.
9. Day JF. Mosquito oviposition behavior and vector control. *Insects* 7: 65, 2016.
10. Donatti JE, Gomes AC. Adultrap: Descrição de armadilha para adulto de *Aedes aegypti* (Diptera, Culicidae). *Rev Bras Entomol* 51: 255-256, 2007.
11. Kumawat R, Singh KV, Bansal SK, Singh H. Use of different coloured ovitraps in the surveillance of *Aedes* mosquitoes in an arid-urban area of western Rajasthan, India. *J Vector Borne Dis* 51: 320-326, 2014.
12. Lima WP, Neto FC, Macoris MLG, Zuccari DAPC, Dibo MR. Estabelecimento de metodologia para alimentação de *Aedes aegypti* (Diptera-Culicidae) em camundongos swiss e avaliação da toxicidade e do efeito residual do óleo essencial de *Tagetes minuta* L. (Asteraceae) em populações de *Aedes aegypti*. *Rev Soc Bras Med Trop* 42: 638-641, 2009.
13. Madeira NG, Macharell, CA, Carvalho LR. Variation of the oviposition preferences of *Aedes aegypti* in function of substratum and humidity. *Mem Inst Oswaldo Cruz* 97: 415-420, 2002.
14. Martínez JM, Rodrigues J, Marreto RN, Mascarin GM, Fernandes ÉKK, Humber RA, Luz C. Efficacy of focal applications of a mycoinsecticide to control *Aedes aegypti* in Central Brazil. *Appl Microbiol Biotechnol* 105: 8703-8714, 2021.
15. Navarro-Silva MA, Marques FA, Duque JEL. Review of semiochemicals that mediate the oviposition of mosquitoes: a possible sustainable tool for the control and monitoring of Culicidae. *Rev Bras Entomol* 53: 1-6, 2009.
16. Nyasembe VO, Teal PEA, Mukabana WR, Tumlinson JH, Torto B. Behavioural response of the malaria vector *Anopheles gambiae* to host plant volatiles and synthetic blends. *Parasit Vectors* 5: 234, 2012.
17. Ponnusamy L, Xu N, Nojima S, Wesson DM, Schal C, Apperson CS. Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. *Proc Nat Acad Sci USA* 27: 9262-9267, 2008.
18. Ponnusamy L, Schal C, Wesson DM, Arellano C, Apperson CS. Oviposition responses of *Aedes* mosquitoes to bacterial isolates from attractive bamboo infusions. *Parasit Vectors* 8: 486, 2015.
19. Reiter P, Amador MA, Colón N. Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. *J Am Mosquito Contr* 7: 52-55, 1991.
20. Rocha LFN, Sousa NA, Rodrigues J, Catão AML, Marques CS, Fernandes ÉKK, Luz C. Efficacy of *Tolypocladium cylindrosporium* against *Aedes aegypti* eggs, larvae and adults. *J Appl Microbiol* 119: 1412-1419, 2015.

21. Rodrigues J, Catão AML, Santos AS, Paixão FRS, Santos TR, Martinez JM, Marreto RN, Mascarin GM, Fernandes ÉKK, Humber RA, Luz C. Relative humidity impacts development and activity against *Aedes aegypti* adults by granular formulations of *Metarhizium humberti* microsclerotia. *Appl Microbiol Biotechnol* 105: 2725-2736, 2021.
22. Roiz D, Wilson AL, Scott TW, Fonseca DM, Jourdain F, Müller P, Velayudhan R, Corbel V. Integrated *Aedes* management for the control of *Aedes*-borne diseases. *PLoS Negl Trop Dis* 12: e0006845, 2018.
23. Rueda LM. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue virus transmission. *Zootaxa* 589: 1-60, 2004.
24. Sant'Ana AL, Roque RA, Eiras AE. Characteristics of grass infusions as oviposition attractants to *Aedes (Stegomyia)* (Diptera: Culicidae). *J Med Entomol* 43: 214-220, 2006.
25. Smith LB, Kasai S, Scott JG. Pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus*: Important mosquito vectors of human diseases. *Pestic Biochem Physiol* 133: 1-12, 2016.
26. Thorn RM, Reynolds DM, Greenman J. Multivariate analysis of bacterial volatile compound profiles for discrimination between selected species and strains *in vitro*. *J Microbiol Methods* 2: 258-264, 2001.
27. Wang Y, Suman DS, Bertrand J, Dong L, Gaugler R. Dual-treatment autodissemination station with enhanced transfer of an insect growth regulator to mosquito oviposition sites. *Pest Manag Sci* 70: 1299-1304, 2014.
28. Weaver S, Scott C, Charlier C, Vasilakis N, Lecuit M. Zika, chikungunya, and other emerging vector-borne viral diseases. *Annu Rev Med* 69: 395-408, 2018.
29. Wong J, Stoddard ST, Astete H, Morrison AC, Scott TW. Oviposition site selection by the Dengue vector *Aedes aegypti* and its implications for Dengue control. *PLoS Neglect Trop D* 5: e1015, 2011.

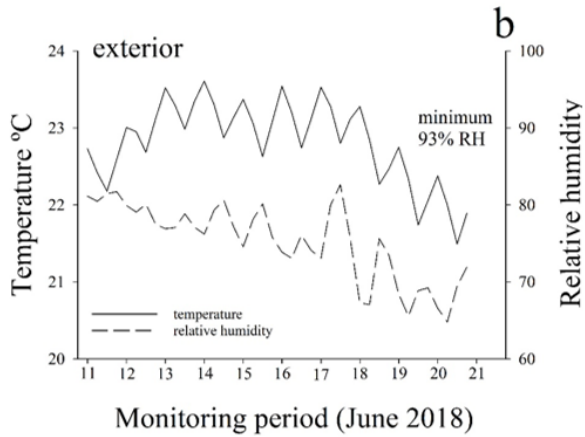
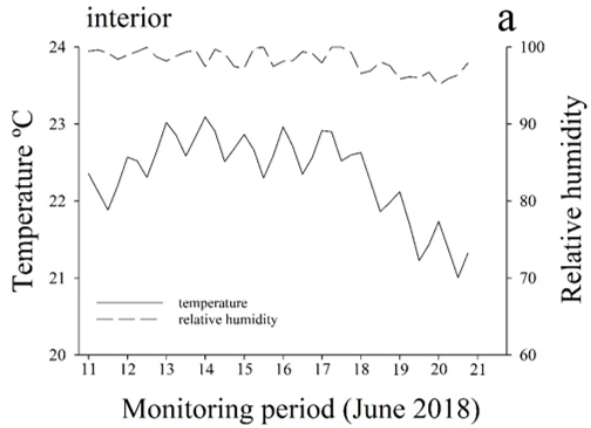


Figure 1S. Monitoring of temperature and relative humidity (RH) inside a disseminating device (a) and in the outside ambience (b) at laboratory conditions (June 11-21, 2018).

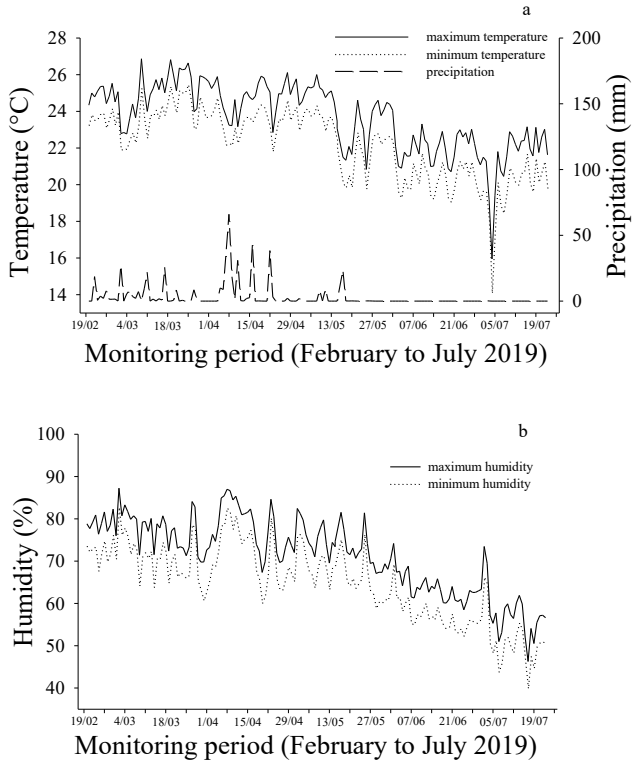


Figure 2S. Daily maximum and minimum temperature (a) and humidity (b), and cumulative precipitation (a) were monitored in the city of Goiânia, Brazil, between February and July 2019 (Meteorological Station, Goiânia A002, OMM 86734).