#### **RESEARCH ARTICLE**



# Occurrence of sweet refuse at disposal sites: rainwater retention capacity and potential breeding opportunities for *Aedes aegypti*

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Received: 27 July 2017 / Accepted: 18 December 2017 / Published online: 6 March 2018 © Springer-Verlag GmbH Germany, part of Springer Nature 2018

#### Abstract

Nectar is the staple diet of adult mosquitoes in the wild, but its availability is inconsistent and can be affected by rainfall. In urban centers, Aedes vectors commonly use man-made containers as their major habitat; however, they can colonize any items replenished by rainfall. Garbage output has increased significantly in recent years, at a time when collection frequency is reducing. Such garbage usually includes organic components, some of which are sweet and can be fed upon by other animals or become can containers for rainwater. Despite evidence that Aedes larvae can thrive in containers comprised of organic waste material, which can be produced by rodents gnawing on fruits or vegetables, and that adults can survive on sweet waste fluids, the capacity of organic waste materials to accumulate rainwater and act as egg deposition sites has not been examined. It is also unknown for how long sweet extracts can sustain the life of adult vectors. Here, we investigated the abundance of sweet leftovers at garbage sites and the rainwater retention capacity of some organic materials through a field survey and laboratory bioassays. We also examined whether sweet waste fluids impact egg hatching success and longevity of Aedes aegypti. The results of this study indicated that sweet products with leftovers are highly prevalent in garbage. When exposed to rain, food items (BAFrc, banana fruit resembling container; and BSPrc, boiled sweet potato resembling container) and the packaging of sweet foods (SMIc, sweetened condensed milk can) retained water. When provided an opportunity to oviposit in cups containing BAF extract (BAFex), BSP extract (BSPex), and SMI extract (SMIex), eggs were deposited in all media. Egg maturation in the BAFex environment resulted in similar larval eclosion success to that resulting from embryo development in a water milieu. Adults maintained on sweet waste extracts had long lifespans, although shorter than that of their sugar solution (SUS)-fed counterparts. Taken together, these results indicated that sweet waste materials are useful to dengue mosquitoes, acting both as oviposition sites and energy sources.

Responsible editor: Philippe Garrigues

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**Keywords** Aedes aegypti  $\cdot$  Garbage  $\cdot$  Sweet waste  $\cdot$  Rain  $\cdot$  Oviposition  $\cdot$  Larval eclosion  $\cdot$  Lifespan

#### Introduction

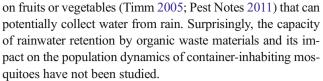
The mosquito, *Aedes aegypti*, is present in many parts of the world and is still a public health problem in developing countries where it is endemic (Weaver and Reisen 2010; WHO/PAHO 2016). This mosquito is a vector of many viral diseases that adversely affect human health worldwide, including Zika virus, which hinders reproductive health, pregnancy, and the developing fetus (Hennessey et al. 2016; CDC 2016), and dengue viruses, which place 2.5 billion people at risk and are responsible for about 24,000 deaths annually (WHO 2013; International Society for Infectious Diseases/ProMEDmail 2017). The ability of this mosquito to persist in these



areas and cause significant public health problems is dependent on its ability to find food and habitats. In general, upon emergence, both sexes of dengue mosquitoes need to feed on sugar for their entire life (Clements 1992; Ignell et al. 2010). Adults that do not have any access to such resources will die quickly (Bellini et al. 2014). Therefore, the availability of sugar sources is critical for disease transmission by these vectors.

Mosquitoes obtain energy in the form of sugar from a number of foods, including homopteran honey dew, aging or damaged fruit, healthy and damaged plant parts, and regurgitates of ants (Foster and Hancock 1994), but mainly nectar (Foster 1995), a complex mixture of many components (Nicolson et al. 2007) with its major ingredient being natural sugar in proportions of around 55% sucrose, 24% glucose, and 21% fructose (Stahl et al. 2012). This staple diet of mosquitoes of both sexes (Yuval, 1992) is not always available or of consistent quality in nature. Nectar is produced by plants mainly within the flowers in glands called nectaries (Galetto and Bernardello 2004). Under conditions of heavy rain, nectar sources become inaccessible (Brown 2017). Both nectar volume and concentration are markedly reduced when flowers are flooded (Tadey and Aizen 2001). In rainy weather, fewer pollen tubes grow in the style than flowers, which reduces accessibility of the nectar (Tadey and Aizen 2001). Heavy rainfall for an extended period can cause flowers to drop (Butt et al. 2015; Maron et al. 2015), and extreme temperatures can delay the timing of flower production and spatial availability of nectar resources (Butt et al. 2015; Maron et al. 2015). Increases in relative humidity reduce nectar secretion (Farkas et al. 2012). These weather-related changes in the availability of nectar may trigger a switch in mosquitoes to use other more readily accessible sugary resources.

Ae. aegypti typically breeds in a multitude of natural and artificial container habitats (Sota et al. 1992; Juliano et al. 2002; Higa 2011). In general, larvae in containers meet their nutritional needs by consuming microorganisms (Walker et al. 1997), particulate matter (Kaufman et al. 1999), or substances released by decomposition of plant material (Dieng et al. 2002). However, these mosquitoes are known to be capable of colonizing any container flooded by rainfall (Liehne 1988), including bottle caps (Roberts 2014) and fruit leftovers (Ferdousi et al. 2015). The amounts of such waste materials generated in developing countries are exceptionally high (UNEP 2005). Such refuse contains mostly organic matter (40-85%) (Hoornweg and Bhada-Tata 2012) and is often left uncollected for considerable periods (Hoornweg and Bhada-Tata 2012). These conditions are conducive to the breeding of both insects, including mosquitoes, and rodents (Hoornweg and Bhada-Tata 2012). In fact, such refuse often contains materials that can accumulate rainwater and become productive breeding sites for dengue vectors (Banerjee et al. 2015). Organic waste attracts rats, which make holes when feeding



In addition to acting as sites for larval development, some sweet leftovers that are prevalent in organic materials (WRAP 2009) can also serve as food sources for adult mosquitoes. Recent evidence indicated that extracts from unfinished cake, banana, and yogurt can successfully support the survival of both sexes of *Ae. aegypti* and reproduction to levels similar to those produced by sugar, their staple food in nature (Dieng et al. 2017). However, it remains unclear for how long they can live when maintained on such sugary fluids. This study was performed to examine the prevalence of sweet waste products at waste disposal sites and the rainwater retention capacity of some sweet waste materials. The oviposition, egg hatching, and longevity responses of *Ae. aegypti* to sweet waste extracts were also assessed.

#### Materials and methods

#### Garbage site survey

A survey was carried out in Kota Samarahan, Malaysia in 2017. Two types of site—one indoor site (the Cais Study Room) and two outdoor sites (Lake View Cafe and Pavillon Restaurant—were surveyed. The garbage sites were examined for the presence of sweet items for four successive days. The surveyor was recommended to inspect for 30 to 45 min per site. All sweet waste products were identified and enumerated. The brand names of all identified sweet materials, the presence/absence of remnants, and residence time were recorded.

#### Testing of sweet waste materials

The foods tested in this study were one fruit (banana), one vegetable (sweet potato), and one dairy product (sweetened condensed milk). The banana was a Bornean cultivar (Family: Musaceae) similar to that used previously (Dieng et al. 2017), also known as honey banana (Pisang Madu in Malay) due to its sweetness. The vegetable was the orange-fleshed cultivar of *Ipomoea batatas* (Family: Convolvulaceae), a seasonal crop grown in tropical and subtropical regions, and an important food source in Asia (International Potato Center 1989). When boiled, a special enzyme breaks down the starch into maltose (Christensen 2009). The sweetened condensed milk (F&N, Sdn Bhd, Malaysia) is a very thick, sweet milk, widely consumed in Southeast Asia (Norimah et al. 2008; Amarra et al. 2016). For convenience, these foods were labeled as BAF, SP, and SMI, respectively.



### Type 1 sweet waste materials and experimental extracts

Volunteers were provided fresh samples of BAF, SP boiled for 10 min (BSP), and SMI cans (SMIc) and asked to eat half of each item and discard the remainder in a garbage bin. After 1-2 h, these uneaten parts were collected and used as experimental sweet waste materials. To produce the different test extracts, we used these type 1 sweet waste materials and followed the procedures reported previously (Dieng et al. 2017). Four replicates of 5 g of BAF pulp were placed in 250-mL plastic containers. After mashing for 2 min, 50 mL of cool water was added to each container and the mixtures were then allowed to disintegrate. After 5 min of soaking, the mixtures were stirred for 5 min and filtered using 60-wire mesh. The resulting solutions were combined and designated as BAF extract (BAFex). The same number of replicates, amount, and operations as reported above for BAF pulp were carried out for BSP flesh and SMI paste, and the resulting solutions were referred to as BSP extract (BSPex) and SMI extract (SMIex), respectively. Two additional diets, water (WAT) and sugar solution (SUS), made by completely dissolving 5 g of sucrose in 50 mL of cool water, were used as a negative control and positive control, respectively.

# Type 2 sweet waste materials and organic waste materials resembling containers

A second group of sweet refuse items was generated. Here, the volunteers were requested to eat 1/10 of the samples of BAF and BSP, and the remaining uneaten parts were disposed of as described above. Upon collection from the garbage bin, the unfinished BAF and SP were provided to caged hamsters that had been fasted for 12 h for 1 or 2 days. The resulting gnawing signs were simulated on other discarded unfinished BAF and BSP. For the milk, cans with 2–3 mL of milk remnants were used. These procedures were repeated when additional experimental sweet waste materials of this type were needed. For convenience, type 2 BAF, BSP, and SMI were designated as BAF resembling container (BAFrc), BSP resembling container (BSPrc), and SMI can (SMIc), respectively.

### Rainwater retention capacity of sweet waste materials

Three sweet waste items were used: BAFrc, BSPrc, and SMIc. To assess the capacity of these sweet waste materials to accumulate water from rainfall, five BAFrc, five BSPrc, and three SMIc in a tray were placed at the same height above the ground in an open area within the premises of the Universiti Malaysia Sarawak on different rainy days. The numbers of rainy days for each of these waste materials were 4, 3, and 3 for BAFrc, BSPrc, and SMIc, respectively. New BAFrc,

BSPrc, and SMIc samples were used for each rainy day. The quantities of retained rainwater collected by the different waste materials were measured at the end of each rainy period.

#### Mosquitoes

Ae. aegypti mosquitoes from a colony maintained under  $28 \text{ °C} \pm 2.0 \text{ °C}$ ,  $75\% \pm 5\%$  (relative humidity), and 13-h light/10-h dark (photoperiod) were utilized in this study. Larvae were reared routinely in plastic trays (As One Corporation, Osaka, Japan) and fed daily with powdered cat food (ProDiet Cat Food, Malaysia). Adults were held in rearing cages  $(30 \times 30 \times 30 \text{ cm})$ , BugDorm; MegaView Science Co., Ltd., Taichung, Taiwan) where they had continuous access to sugar diet (10% sucrose solution). For maintenance, females were routinely blood fed on hamsters, two to three times per month, 4–5 days after emergence. Eggs dried in the room environment for 1 week were used as stock colonies.

### Egg laying responses by Ae. aegypti in sites with sweet waste extracts

To examine whether *Ae. aegypti* females will lay eggs in sweet waste water, five females (5–6 days old) that were gravid (i.e., had taken and digested blood for 3 days) were placed in a mosquito cage (30 × 30 × 30 cm) with an oviposition device. The device consisted of circular dish with two cups (acrylic container, depth = 7.3 cm, diameter = 3.3 cm) containing a piece of filter paper (length = 8 cm, width = 8 cm). To the cups were added 30 mL of one of the subsequent media: (i) water (control) and (ii) BAFex. Four extra replicates of the treatment (one cup with water + one cup with BAFex + five gravid females) were set up on the same or different days. The same treatment was also performed for BSPex and SMIex with five replicates in each case. After a 3-day oviposition period, cups were checked for the presence or absence of eggs in all bioassays.

#### Sweet extracts and egg hatching success

To determine whether the sweet waste extract-based maturation milieu affects larval eclosion success, all eggs in the oviposition study that were laid on the substrates submerged in the different test media (WAT, BAFex, BSPex, and SMIex) were allowed to air-dry for 3 days. The dried eggs that completed development under each of these environments were divided into groups of 15–30 eggs each (WAT, six groups; BAFex, eight groups; BSPex, 15 groups; and SMIex, six groups). Each of these groups was flooded in acrylic vials holding 25 mL of cool boiled tap water, as described previously (Saifur et al. 2010). Vials were inspected after 24 h, and egg hatching responses were recorded.



#### Sweet extracts and longevity success

To obtain experimental adults, samples of eggs from the maintenance colony were flooded in tap water. Newly hatched larvae were reared in quadruplicate 4-L plastic trays at a density of 250 and supplied with 0.15 g of ProDiet cat food according to the feeding schedule described previously (Dieng et al. 2015). Four groups of 15 females that emerged on the same day were each placed in mosquito cages (30 × 30 × 30 cm) where they had access to one of the following food sources: WAT, SUS, BAFex, BSPex, or SMIex. Similar numbers of groups, numbers of individuals per group, and feeding regimes as outlined for females were also set up for males of the same age at emergence. All ten cages (five for females and five for males) were maintained under conditions of controlled temperature (28 °C ± 2.0 °C), relative humidity  $(75\% \pm 5\%)$ , and a photoperiod of 13 h light/10 h dark, checked on a daily basis, and numbers of deaths (if they had occurred) were recorded. Each experiment concerning a given sex was terminated when the last individual died.

#### **Data collection and analysis**

At each garbage site, the sweet waste materials found were identified according to type and brand and counted. The number of items with food remains and the number of days present at a given garbage site were also noted. Sweet waste abundance at the different sites were determined based on percentages (number of sweet items found at a given site) / total number of sweet items collected during the survey) × 100. The prevalence of food remnants was defined as the number of sweet items found with remains at a given site) / total number of sweet items found) × 100. The number of days spent at a garbage site was scored as the residence time. In the rainwater retention experiment, the volume of water collected by each replicate of each of the exposed items was recorded after each rainfall, and these numbers were used to calculate the mean (± SE) values that were used as scores of rainwater retention capacity. In the egg hatching bioassay, an oviposition response to a given sweet waste-derived medium was based on the presence of at least five eggs on the substrate or the surface of the medium determined using a dissecting microscope (Meiji EMZ; Meiji Techno Co., Ltd., Tokyo, Japan). In the egg hatching bioassay, the number of hatched eggs was determined for each group with each medium by counting the numbers of first-instar larvae found in vials after the 24-h submersion period. These numbers were used to calculate egg hatching rates as the number of hatched eggs divided by the total number of eggs initially flooded × 100; the resulting mean ( $\pm$  SE) values were considered as egg hatching success, as reported elsewhere (Satho et al. 2015). In the lifespan bioassay, dead individuals were enumerated for each sex. The mean ( $\pm$  SE) times in days between larval eclosion and the death of adults were used as measures of lifespan. The discrepancies in the abundance of sweet waste items and the prevalence of those with food remnant prevalence between sites were compared based on percentages. In the mosquito–sweet waste interaction bioassays, differences in the parameters examined were compared by analysis of variance (ANOVA) using Systat v.11 statistical software (Systat Software, Inc. 2004) where appropriate. Tukey's honestly significant difference (HSD) test was used for separation of mean ( $\pm$  SE) values. In all analyses, P < 0.05 was used as proxy of statistical significance.

#### Results

### Diversity and abundance of sweet waste products in waste disposal sites

A total of 13 sweet items—23.07% (3/13) from the indoor site (Cais Study Room), 46.15% (6/13) from the Lake View Café (outdoor site), and 30.78% (4/13) from the restaurant (outdoor site)—were found. The presence of food remnants was high at all garbage sites: 100% outdoors (Lake View Café and Pavillon Restaurant) and 66.6% indoors (Cais Study Room). Garbage was not removed on a daily basis, and the residence times of most identified waste items ranged between 1 and 3 days (Table 1).

### Rainwater retention capacity of sweet waste materials

Both food items (BAFrc and BSPrc) and sweet food packaging (SMIc) retained water after rainfall, but the amounts accumulated varied with product size. Visually, the SMIc was large, BAFrc was small, and BSPrc was intermediate in size. The amount of rainwater collected was much higher for SMIc than BAFrc. It is likely that the juice generated in BAFrc was more concentrated than those from the larger materials (SMIc and BSPrc), and it is therefore likely that the BAF juice was sweeter (Table 2).

### Egg laying responses by Ae. aegypti in sites with sweet waste extracts

When provided with equal chances to lay eggs in a container holding water and another with sweet waste extract (BAFex, BSPex, and SMIex), *Ae. aegypti* deposited eggs in all media, suggesting that females are attracted to containers with sweet waste extract (Table 3).



Table 1 Characteristics of sweet waste materials found at different garbage sites in Kota Samarahan (Malaysia) in April 2017. Plus (+) and minus (–) indicate the presence and absence, respectively

Garbage location	Sweet waste type	Food remnant	Residence time (days)	
Indoors (Cais Study Room)	Drink can	+	3	
	Jelly container	+	3	
	Appolo cake	_	2	
Outdoors (Lake View Cafe)	Drink can	+	2	
	Cardbury pack	+	2	
	Chocolate	+	2	
	Ice cream cup	+	2	
	Kinder Brunos	+	2	
	Bubble gum	+	1	
Outdoors (Pavillon Restaurant)	Milo drink	+	2	
	Drink can	+	2	
	Syrup drink pack	+	2	
	Ice pop	+	2	

# Egg maturation in sweet waste environments and hatching responses

Egg hatching responses varied considerably according to the conditions under which the embryos developed (F =11.481; df = 3; P < 0.001). The mean number of eggs that hatched following maturation on substrates imbibed with WAT was  $34.16\% \pm 9.43\%$ . The maintenance of newly laid eggs in BAFex and SMIex milieu resulted in mean hatching success rates of  $31.42\% \pm 8.14\%$  and  $5.83\% \pm 2.38\%$ , respectively. No eggs hatched when maturation occurred in the BSPex environment. Tukey's test indicated that egg hatching success after embryo development in a WAT milieu was similar to that produced by egg maturation in the BAFex environment (Matrix of pairwise mean differences (MPMD = -6.667; P = 0.829)). Egg hatching responses obtained after egg maturation in the SMIex environment was significantly lower than those produced by WAT (MPMD = -28.333; P = 0.010) and BAFex (MPMD = -21.667; P = 0.044) conditions (Fig. 1).

**Table 2** Mean  $(\pm\,SE)$  volumes (ml) of rainwater retained by the different sweet waste materials. BAFrc: banana fruit resembling container; BSPrc: boiled sweet potato resembling container; SMIc: sweet milk can

Rain	Sweet waste material					
	BAFrc	BSPrc	SMIc			
One	$0.58 \pm 0.24$	$1.45 \pm 1.01$	$173.00 \pm 7.57$			
Two	$0.64 \pm 012$	$12.2\pm1.93$	$456.66 \pm 3.33$			
Three	$0.87 \pm 037$	$7.12\pm3.28$	$10.96 \pm 0.57$			
Four	$0.36 \pm 025$	$2.95 \pm 0.74$	$11.37 \pm 0.42$			

#### Lifespans of females maintained on waste-based diets

The mean lifespans of Ae. aegypti females maintained on WAT and SUS were  $18.57 \pm 0.37$  (range 17-21 days) and  $55.13 \pm 4.05$  days (range 19–78 days), respectively. In the sweet waste extract treatments, these values were  $30.06 \pm$ 2.68 (range 13–45 days),  $26.73 \pm 1.99$  (range 19–39 days), and  $34.28 \pm 2.78$  days (range 19–49 days) for BAFex, BSPex, and SMIex, respectively. Diet type significantly affected longevity (F = 27.009; df = 4; P < 0.001). Females maintained on WAT had significantly shorter lifespans than their counterparts fed BAFex (MPMD = 11.495; P = 0.028), which in turn had a similar lifespan to BSPex-fed females (MPMD = -5.221; P = 0.662). These latter females (BSPexfed) tended to have shorter lifespans than those fed SMIex, but the difference in lifespan between these two types of females was not significant (MPMD = 9.440; P = 0.129). SUS-fed females lived far longer than their WAT-fed counterparts (MPMD = 36.562; P < 0.001) or those fed SMIex (MPMD =20.848; P < 0.001) or any of the other sweet waste extracts

**Table 3** Oviposition responses of *Ae. aegypti* when given opportunities to lay eggs in containers with water and different sweet waste extracts. WAT: water (negative control); BAFex: banana fruit extract; BSP extract: boiled sweet potato extract; SMIex: sweet milk extract

Trail	Medium in oviposition site						
	WAT	BAFex	WAT	BSPex	WAT	SMIex	
1	+	+	+	+	+	+	
2	+	+	+	+	+	+	
3	+	+	+	+	+	+	
4	+	+	+	+	+	+	
5	+	+	+	+	+	+	



(P < 0.001). The mean lifespans of females fed BAFex, BSPex, and SMIex were 1.61, 1.43, and 1.84 times that of WAT-fed females, respectively (Fig. 2A).

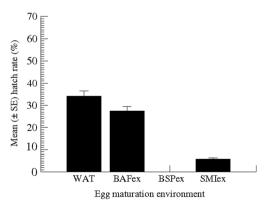
#### Lifespans of males maintained on waste-based diets

WAT- and SUS-fed males lived for  $16.20 \pm 0.32$  (range 15-19 days) and  $38.60 \pm 4.46$  days (range 15-61 days), respectively. Males showed lifespans of  $18.73 \pm 1.24$  (range 13-29 days),  $18.06 \pm 0.70$  (range 13-21 days), and  $21.13 \pm 1.95$  days (range 15-45 days) when maintained on BAFex or BSPex and SMIex, respectively. Male lifespan varied significantly with diet (F=16.117; df=3; P<0.001). The mean lifespan of WAT-maintained males was similar to those of BAFex-fed (MPMD=2.533; P=0.934), BSPex-fed (MPMD=1.867; P=0.978), and SMIex-fed males (MPMD=4.933; P=0.546), but these were all far lower than that of males maintained on SUS (MPMD=22.400; P<0.001) (Fig. 2B).

#### Discussion

The results of the present study indicated that leftover sweet products are prevalent in garbage sites, and that all of the sweet waste resembling containers accumulated rainwater. Ae. aegypti females deposited eggs when provided with cups containing sweet waste media when in balanced competition with water. Egg hatched following maturation in sweet wastemoistened environments, in some cases with a success rate similar to that seen with maturation in water milieu. We also found increases in lifespan among females and males maintained on sweet waste extracts compared to sugar, their staple food in nature (Gary and Foster 2004).

The present survey performed both outdoors and indoors revealed an increased prevalence of sweet waste items with



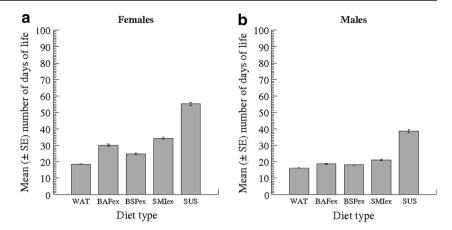
**Fig. 1** Hatching responses of *Ae. aegypti* eggs that matured on substrates soaked with WAT (control) or with various sweet waste solutions. BAFex, BSPex, and SMIex: banana, boiled sweet potato, and sweet milk extracts, respectively



remnants and infrequent collection (the majority of these materials remained uncollected for 2-3 days). An increased residence time of waste outdoors will tend to result in higher likelihood of rodent gnawing activity (Timm 2005), which will produce holes in fruits, vegetables, and packaging (Pest Notes 2011). In the present study, holes with different opening sizes were observed on potatoes, bananas, and apple parts placed in cages with hamsters for 2 days. These consequences of rodent feeding activities were used as a methodological approach in our rainwater retention study where exposure of container-like waste materials (BAFrc, BSPrc, and SMIc) resulted in the retention of appreciable volumes of rainwater. In nature, dengue vectors can breed in any container habitat flooded with rainwater (Liehne 1988; Higa 2011), including both phytotelmata (i.e., water held by terrestrial plants) and man-made containers (Sota et al. 1992) with amounts of water as small as that held in a bottle cap (Baldacchino et al., 2015). In Kenya, rain-filled fruits were reported to be highly attractive to ovipositing mosquito females and suitable for completion of larval development (Lounibos 1978). In India, the high prevalence of cut fruits across cities has been suggested to be responsible for the increased incidence of dengue in this country (Hindustan Times 2012). In Bangladesh, discarded coconut shells were found to be infested with larval Aedes albopictus and Ae. aegypti (Ferdousi et al. 2015), indicating egg deposition in these containers. The observed accumulation of rainwater by BAFrc, BSPrc, and SMIc in the present study strongly suggests that they are potential breeding habitats for Aedes mosquitoes. Consistent with this suggestion, we found that gravid Ae. aegypti readily oviposited when provided with cups containing extracts from BAF, BSP, and SMI.

In container-breeding mosquitoes, habitat productivity has often been associated with water permanence (Bradshaw and Holzapfel 1983; Tsuda et al. 1994) and availability of food sources (Arrivillaga Barrera 2004; Mogi 2010). There are close links between amount of water, habitat permanence, and oviposition. Indeed, habitats with sufficient water will tend to be more permanent and attractive to gravid females. Therefore, SMIc would likely be inviting sites for egg deposition as they can collect up to 450 mL of rainwater. Food source availability (Arrivillaga and Barrera 2004; Mogi 2010) also plays a major role in determining habitat suitability. Indeed habitats with more nutrients will increase the likelihood that larvae will develop successfully and quickly. Some entomologists (Clements 1963; Fish and Carpenter 1982) reported that detrital particulate matter and associated microbes represent major portions of larval aedine diets (Kaufman et al. 1999; Satho et al. 2015). With the presence of water, particulates are more subjected to detachment from both the banana pulp and sweet potato flesh, which may act as nutritional resources. The widely open aspect and the permanence of water of SMIc are also likely to have an impact on mosquito larval food availability. Large openings are often

Fig. 2 Mean lifespans (± SE) of *Ae. aegypti* adults (females and males) provided equal opportunities to feed on various diets. WAT: water (negative control); BAFex: banana fruit extract; BSP extract: boiled sweet potato extract; SMIex: sweet milk extract; SUS: sugar solution (positive control)



associated with abundance of detritus in container habitats, because they can collect falling leaves, which will further decompose into particulate matter that is known to be directly ingestible by *Aedes* larvae (Kaufman et al. 1999).

The eggs laid by Ae. aegypti females that matured in BAFex hatched at rates similar to those in a water environment. Eggs maintained in SMIex-soaked substrates hatched at a low rate. In contrast, none of the eggs maintained in BSPex hatched, indicating that the presence of vegetable extract affected egg viability. For an aedine egg to be viable, the embryo must retain sufficient moisture during development (Strickman 1980), while losing water via osmosis (Rezende et al. 2008; Vargas et al. 2014). The quality of water uptake depends largely on the surrounding environment and its ionic balance. Aedine eggs absorb sufficient amounts of water and hatch readily when maintained on water-moistened paper, they but are nonviable when they matured in an oily environment (Rosay 1959). In a related study, Satho et al. (2015) found that larval eclosion rate is low when Ae. aegypti eggs are reared on paper substrates moistened with coffee compared to those kept on water-soaked substrates; they suggested that the high ionic balance in the coffee milieu affects water transfer into the eggs. The also suggested that coffee altered the cuticle tanning/hardening process by repressing the activity of DOPA decarboxylase, an enzyme necessary for synthesis of sclerotizing agents in insects (Karlson and Sekeris 1962). In dengue mosquito eggs, DOPA decarboxylase levels are high in viable eggs (Li et al. 1996) and low in those that are nonviable (Xue et al. 2005). In the present study, the maintenance of eggs on substrates soaked with BSPex resulted in complete hatching failure. With reference to the reports mentioned above, it is likely that some substances in BSPex altered water transfer, thus resulting in insufficient water uptake into the eggs. It is also likely that some BSPex compounds acted as inhibitors of DOPA decarboxylase. Further investigations are necessary to clarify the anti-egg hatching potential of BSPex.

Ae. aegypti mosquitoes of both sexes maintained exclusively on the tested sweet waste extracts showed long

lifespans. Female lifespan reached about 30, 26, and 34 days when maintained on BAF, BSP, and SMI extracts, respectively; these values were 21 and 18 days for males maintained on SMIex and BAFex or BSPex, respectively. Adults maintained on WAT shoed much shorter lifespans than those fed sweet waste extracts. Taken together, these observations indicated that Ae. aegypti met their sugar requirements when sweet waste extracts were available as food sources. The sugar contents of discarded sweet waste have been reported for many products. Fruits naturally contain fructose (Park and Yetley 1993; Riby et al. 1993), glucose (Ushijima et al. 1991), and sucrose (Buss and Robertson 1976), and sucrose has also been reported to be present in some root vegetables (Buss and Robertson 1976). Bananas contain 12.2 g of sugar per 100 g (NAL USDA 2016). Yogurts are rich in the milk sugar, lactose (Kimball, 2012), as well as added sweeteners (Walker and Goran 2015). Sweet potatoes are rich in starch and sugars, including maltose, sucrose, and glucose (Picha 1985, 1986; Lai et al. 2013). In fact, females of many mosquito species, including aedine species, caught in the field have been shown to contain these diverse types of sugar (Burkett et al. 1998). In the present study, extracts were produced by mashing 5 g of material or condensed milk in 100 mL of water and allowed to disintegrate for 5 min prior to stirring for 3 min. All three extracts (BAFex, BSPex, and SMIex) tasted sweet at the end of the production processes. These observations support the assumption that sugary components were released from BAFex, BSPex, and SMIex, which may have been ingested by both sexes of Ae. aegypti at different levels. The level of disintegration of submerged material is influenced by the texture of the composing matter. Generally, the harder the flesh the smaller the amount of labile substances released. Therefore, a plausible explanation for the discrepancies between BSPex- and SMIex-fed females is that BSPex released much lower amounts of sugary components than the two other extracts tested (BAFex and SMIex). The slightly longer lifespans of SMI-maintained females compared to their BAF-fed counterparts was likely because SMIex contained more sugary substances than the BAF fluid, and it is clear that



the SMI paste dissolved more quickly that BAF pulp. Milk consists of proteins, fat, salts, and the milk sugar, lactose (Fox 1995), and sweet milk has up to 45% added sugars (Goff 1995, Silverson Machines Inc. 2015). Banana contains fiber, sugars (16% fresh weight), and very small amounts of protein and fat (Zhang et al. 2005). Several anti-oxidant flavonoids (Someya et al. 2002), caffeic acids (Ongphimai et al. 2013), and alkaloids (Ehiowemwenguan et al., 2014) are present in banana. These latter compounds were reported to inhibit feeding (Araque et al., 2007) or bioassimilation (Mitchell et al. 1993) in insects. It is also possible that the observed discrepancies in female lifespan between BAFex and SMIex regimes were due to differences in sugar consumption. There may also be direct effects of BAF anti-feedant compounds that interfere with adult feeding activity, which would reduce sugar intake and decrease lifespan. This may not be the case in the MI regime. The relatively long lifespans obtained with BAFex, BSPex, and SMIex could have important epidemiological implications. With a prolonged adult lifespan, females may mate more, take more blood meals, transfer or pick viruses more frequently, and lay more eggs. This would therefore result in a large adult population size and an increased likelihood of disease incidence.

#### **Conclusions**

Our findings indicated that garbage sites are abundant in sweet leftover items, which are left exposed and uncollected for 2-3 days. Such conditions are likely to be the case in many parts of Southeast Asia where food waste generation is high (Gustavsson et al. 2011; Nation 2016; Curea 2017) and waste management is insufficient (Gregory et al. 1996; da Silva 2016). In addressing the issue of solid waste management and infectious diseases, Hoornweg and Bhada-Tata (2012) suggested that uncollected refuse has the potential to provide breeding sites and food resources to insect vectors. This was corroborated by the observations in the present study: overall, our results illustrated that sweet waste materials can accumulate rainwater and the resulting fluids can act as both oviposition media allowing increased embryo viability (at least BAFex) and as an energy source that can maintain adult vector life for up to 1 month. These attributes associated with the increased amounts of sweet waste materials (Gustavsson et al. 2011; Jacobi and Besen 2011; Nation 2016; Curea 2017) and positive effects on biting as well as egg production (Dieng et al. 2017) suggest that sugary waste items contribute to dengue and Zika virus disease outbreaks in Asia and Latin America, a region where garbage collection is infrequent (López 2014). Solid waste generation, in particular organics, is an increasingly significant environmental and public health problem in Asian urban centers (Pariatamby and Tanaka 2013; da Silva 2016) with about 760,000 t produced daily, with further increases related to rapid economic growth expected in the future leading to a projected amount of 1.8 million t/day by 2025 (Curea 2017). With this waste output and the informal recycling sector and poorly coordinated waste management policies (Curea 2017), public health issues, including Aedes-borne illnesses, will likely increase in magnitude. Several strategies can be adopted to overcome these potential epidemiological problems. This organic waste-Aedes association is similar to the accumulation of discarded tires reported in the USA (Baumgartner 1988). This author investigated the impacts of outdoor tire storage on rainwater retention and mosquito colonization and reported that despite the small size of their aggregations and cryptic nature, tires contributed significantly to the population densities of many mosquito species, including dengue vectors. One particular similarity with our study is the analysis of rainwater accumulation. In the present study, we observed small levels of rainwater accumulation by the food items (BAFrc and BSPrc) and larger levels in the sweet food packaging (SMIc), potentially representing highly concentrated sugar sources and larval development media, respectively. Baumgartner suggested that awareness campaigns alerting the public to improper waste management and health hazards of mosquitoes, frequent removal, disposal site roofing to prevent rainwater accumulation, and application of insecticides to garbage sites can help to prevent the potential mosquito problems associated with uncollected waste. Similar approaches can be applied to garbage disposal sites. In addition, making drainage holes in any organic waste material resembling containers, such as cut fruits (coconut shells, peels of mango, watermelon, and durian), cans, yogurt, or ice cream cups, and slicing or cutting uneaten fruits or vegetables into small pieces prior to discarding can potentially prevent rainwater accumulation and therefore reduce the generation of sugary fluids.

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