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RESEARCH ARTICLE

Coffee, its roasted form, and their residues cause birth failure and shorten lifespan in dengue vectors

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Abstract In dengue mosquitoes, successful embryonic development and long lifespan are key determinants for the persistence of both virus and vector. Therefore, targeting the egg stage and vector lifespan would be expected to have greater impacts than larvicides or adulticides, both strategies that have lost effectiveness due to the development of resistance. Therefore, there is now a pressing need to find novel chemical means of vector control. Coffee contains many chemicals, and its waste, which has become a growing environmental concern, is as rich in toxicants as the green coffee beans; these chemicals do not have a history of resistance in insects, but some are lost in the roasting process. We examined whether exposure to coffee during embryonic development could alter larval eclosion and lifespan of dengue vectors. A series of bioassays with different coffee forms and their residues indicated that larval eclosion responses of *Aedes albopictus* and *Ae. aegypti* were appreciably lower when embryonic maturation occurred in environments containing coffee, especially roasted coffee crude extract (RCC). In addition, the lifespan of adults derived from eggs that hatched successfully in a coffee milieu was reduced, but this effect was less pronounced with roasted and green coffee extracts (RCU and GCU, respectively). Taken together, these findings suggested that coffee and its residues have embryocidal activities with impacts that are carried over onto the adult lifespan of dengue vectors. These effects may significantly reduce the vectorial capacity of these insects. Reutilizing coffee waste in vector control may also represent a realistic solution to the issues associated with its pollution.

Keywords Coffee · Coffee waste · Dengue vector · Embryonic development · Adult lifespan

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Introduction

With over 40% of the world's population at risk (WHO 2012a), 100 million cases annually, and 1 in 2000 cases resulting in death (CDC 2015), dengue has become the most common arboviral disease. Management of this disease has been attempted using a variety of methods, mostly targeting vector populations with chemical insecticides (WHO 2012a). Some such programs have worked well (Ooi et al. 2006), but the two main vectors, *Aedes aegypti* (Dia et al. 2012) and *Ae. albopictus* (Vontas 2012), have acquired resistance to the four classes of insecticides registered for vector control. With the absence of licensed vaccines and treatments (Laughlin et al. 2012), the ongoing reductions in availability of effective insecticides due to resistance, and increased anti-pesticide

activism and prohibitions on their use, there is a need for the development of new chemical means of vector control.

Approximately 500 billion cups of coffee are consumed worldwide annually (Schermerhorn 2012). In general, coffee drinks are made by brewing hot water with ground roasted beans or by immersing bags containing ground particles in boiled water. These coffee grounds are usually discarded after brewing (Gutierrez 2015). Most of the 8 billion kilograms of coffee grown annually worldwide ends up as waste (Gutierrez 2015). The by-products of roasting coffee beans and brewing coffee amount to over 2 billion metric tons annually (Jiménez-Zamora et al. 2015), which represents a global environmental issue (Kennedy 2013). As many coffee-producing countries are attempting to expand production (The Economic Times 2015) due to increased demand (Bariyo 2015), the management of coffee waste is still a major challenge (Leibrock 2014) despite the availability of many recycling options (Chalker-Scott 2009).

The popularity of coffee is chiefly due to its high levels of bioactive compounds that have been shown to exert health benefits (Paquin 2009). These beneficial attributes of coffee are independent of its caffeine content (Huntington 2014). Coffee contains more than 1000 bioactive constituents, including a complex mixture of polyphenols (Clarke 2013). The most abundant polyphenol, chlorogenic acid (Preedy 2014), has important beneficial effects against heart problems (Meng et al. 2013). Although millions of people enjoy drinking coffee every day (Santo and Lima 2009), it is harmful to some animals, including insects. For example, the uptake of caffeine alters embryogenesis in flies (Itoyama et al. 1992). This substance depresses feeding in beetles (Araque et al. 2007), impedes the webbing capacity of spiders (Foelix 2010), and kills some other insects (Nathanson 1984). Polyphenolic compounds, such as chlorogenic acid, reduce food consumption and assimilation in moths (Mallikarjuna et al. 2004), cicadellids (Dowd and Vega 1996), and plant lice (Miles and Oertli 1993). Coffee alkaloids, particularly caffeine, are lethal to many insect species at high doses (Nathanson 1984; Wink 1992). In flies, coffee affects oviposition rate, egg development, and adult longevity (Itoyama et al. 1998). In dengue mosquitoes, exposure of eggs, larvae, or adults to either caffeine or coffee was reported to alter reproductive traits and longevity (Laranja et al. 2003) and oviposition behavior (Satho et al. 2015).

In addition to the diversity of physiological effects, the possibility that coffee may be used for management of mosquito-borne diseases is supported by its variability in chemical contents. Various factors, such as coffee plant cultivar (Semmelroch and Grosch 1996), soil type, weather, agricultural techniques, storage conditions (Farah et al. 2006; Perrone et al. 2008), maturation level (Farah 2012), temperature, and time of roasting (Perrone et al. 2012), influence the chemical composition of coffee. The dimension of the coffee grounds, brewing conditions, and extraction time as well as water impacts the chemical profile of brews (Farah 2012). The green coffee beans mainly contain chemical precursors (Holscher and Steinhart 1995), and it is the roasting process that produces the chemical richness of coffee (Fisk et al. 2012).

There has been a great deal of research effort into testing the effects of coffee against dengue vectors (Laranja et al. 2003; Derraik and Stanley 2005); however, most of these studies focused on larvae and did not investigate their eggs. In dengue vectors, this stage has great potential as a target for control measures (Ezeakacha 2015) as it is a prerequisite for the production of subsequent generations (Dieng et al. 2006) and can spread the viruses (Thenmozhi et al. 2007). To address dengue vector control issues, the World Health Organization recommends that management strategies should take eggs into account (Sarkar 2010). Immediately after oviposition, the egg is composed of two layers, the endochorion and the exochorion (Clements 1992). During embryonic development, a third layer, the serosal cuticle, gradually appears between the two other layers (Beckel 1958). These structural changes protect the embryo (Morris 1997) and lead to a progressive decrease in permeability (Harwood and Horsfall 1959). In the main dengue vector, *Ae. aegypti*, the hardening process of the egg occurs much earlier than acquisition of the serosal cuticle (Vargas et al. 2014). Chemical agents can penetrate the cuticular barrier of the eggs of dengue mosquitoes, which can impair their viability (Luz et al. 2008). As the developing embryo is prone to water loss (Vargas et al. 2014), this may minimize the toxic effects of chemical agents. However, sublethal responses of embryos to insecticides and their effects on adult life history traits remain largely unexplored (Stoks and Córdoba-Aguilar 2012). One such trait that can impact the general transmission route and vectorial capacity is the lifespan of an adult female mosquito (Macdonald 1957). After taking a blood meal from a dengue-infected person, the ingested virus requires a period of development within the female; the virus cannot develop if the female does not survive the incubation period (Clements 1992; Goindin et al. 2015). Many authors have concluded that mosquito lifespan is the most important factor affecting vectorial capacity (Costanzo et al. 2015). Macdonald (1957) reported that even small changes in this factor can greatly impact the spread of disease. Joy et al. (2010) also suggested that reducing the lifespan of old mosquitoes that transmit dengue could decrease the overall level of dengue transmission. Some entomologists (Laranja et al. 2003) have examined the effects of caffeine with mosquito eggs and used coffee grounds to alter the lifespan of dengue mosquitoes, but they did not examine the effects of green coffee beans. Unlike caffeine, which must be purified from coffee, green coffee beans are commonly available in coffee-producing countries, many of which are located in tropical areas. Surprisingly, the authors also ignored

Ae. albopictus. This species has replaced and is still replacing *Ae. aegypti* in many tropical areas (Enserink 2008; Kamgang et al. 2013) and has been implicated in recent outbreaks of both dengue and chikungunya (Rezza 2012; Vega-Rúa et al. 2014). In the present study, we examined the effects of exposing embryos of two dengue vectors, *Ae. aegypti* and *Ae. albopictus*, to four types of coffee preparation (roasted, fresh, and their used forms) on larval eclosion and adult lifespan.

Materials and methods

Mosquito colonies

This study was performed using *Ae. albopictus* and *Ae. aegypti* colonies initiated from larvae and pupae collected from across Penang Island, Malaysia. Insects were maintained under conditions of controlled temperature (29 ± 3.0 °C), relative humidity (75% ± 5%), and a photoperiod of 13 h light/10 h dark from two fluorescent tube lights (80 W). Larvae (100–150) were reared in metallic containers (radius = 6 cm, depth = 2 cm) containing 1 L of dechlorinated water. Larval feed (a blend of dog biscuits, beef liver, yeast, and milk powder in the ratio (*w*/*w*) 2:1:1:1) was given during larval development as follows: 1–2, 3–5, and 5–7 mL of larval food suspension (0. 15 g/4 mL) on day 1 (day of egg hatching) and on days 3 and 5 post-hatching. Pupae were collected from rearing containers every 2 days and placed in cups (250-mL capacity) that were later transferred in standard adult mosquito breeding cages (30 × 30 × 30 cm). Adults had continuous access to 10% sucrose solution, and females were given blood meals from immobilized mice on days 4–5 post-emergence. After 3 days of blood digestion, eggs were collected, dried, and kept as an egg bank (Dieng et al. 2014).

Experimental subjects

To generate experimental adults, samples of *Ae. albopictus* eggs from colonies laid 3 weeks earlier were flooded with dechlorinated water. On the following day, 150 newly hatched larvae were reared in quadruplicate in containers similar to those described earlier holding 1 L of water. For all four replicates, larvae were fed the same larval food suspension used for colony maintenance in accordance with the feeding regime of Satho et al. (2015). Briefly, the amounts administered were as follows: 3 mL on hatching day and 6 mL on days 3, 5, 7, and 9 post-hatching. Pupated individuals were transferred singly into containers holding 0.5 mL of tap water. Adults were checked for gender following emergence. Males from all replicates were transferred to a standard adult mosquito cage and provided 10% sucrose solution from a cotton wick. Females were also grouped in another cage and provided with the same treatments as for males. Females 4–5 days old were blood fed on retrained mice, and fully engorged mosquitoes were transferred into another cage with access to 10% sucrose solution. Identical numbers of replicates, larval density, and feeding regimes (for larvae and adults) as well as adult treatment (sex separation) outlined above for *Ae. albopictus* were also performed for *Ae. aegypti*.

Experimental coffee strain and experimental extracts

A high-grade *Coffea canephora* variety grown in Kedah State (Malaysia) was selected for this study. Roasted and dried green seeds were separately crushed using a blender (Pensonic Blender PEN-PB3103; Senheng® Electric Sdn. Bhd., Kuala Lumpur, Malaysia) and sieved through a 60-wire mesh. The different extracts were generated following a slight modification of the procedures described previously (Satho et al. 2015). Briefly, five replicates of 9.4 g of ground roasted coffee (RC) were added to 250-mL glass containers holding 150 mL of freshly boiled water. One hour after immersion, the brews were filtered using fine-mesh mosquito nets, and the remnants were transferred into new glass containers. The five extracts were pooled and considered as RC crude extract (RCC). Freshly boiled water (150 mL) was added to each of the five remnants and allowed to decay for 1 h, and the pooled filtered extracts were referred to as RC used extract (RCU). The same amount of coffee, number of replicates, and operations described above for RC were repeated but with green coffee (GC); the two resulting sieved solutions were referred to as GC crude extract (GCC) and GC used extract (GCU), respectively (Table 1). GC was used for scientific comparison against RC, which is the target sample to be studied.

Experiments

Twenty-five virgin 2–5-day-old males of *Ae. albopictus* were placed in a cage (30 × 30 × 30 cm) and allowed to acclimatize. After 10 min of acclimatization, 3–4-day-old females were released into the cage in groups of two or four individuals. Five other replicates of the same number of males and females with ages similar to those of their counterparts mentioned above were set up. These cohorts were individually observed for copulation, and any pairs that copulated for at least 5 s were collected using an aspirator. The males were discarded and copulated females were transferred into a new cage. After 10 min of habituation, females were given blood feeding opportunities by placing a restrained mouse within the cage. After 1 h of exposure, the blood source was withdrawn and females were examined for blood meal uptake. Fully engorged females were transferred to a new cage and permitted to digest the blood meal. After 3 days of blood meal digestion, females that were considered to be gravid were individually transferred into oviposition apparatuses identical to those described

Table 1 Production of experimental coffee extracts used in this study

Amount	Brewing procedures	Extract
9.4 g of roasted grounds	1. First extraction ►Dissolution in 150 mL of boiled water ►1-h extraction period ►Brew filtration	Roasted coffee crude extract (RCC)
	2. Second extraction ►Dissolution in 150 mL of boiled water ►1-h extraction period ►Brew filtration	Roasted coffee used extract (RCU) Fresh coffee crude extract (GCC) Fresh coffee used extract (GCU)
9.4 g of green grounds	1. First extraction ►Dissolution in 150 mL of boiled water ►1-h extraction period ►Brew filtration	
	2. Second extraction ►Dissolution in 150 mL of boiled water ►1-h extraction period ►Brew filtration	

previously (Dieng et al. 2013). Briefly, the oviposition apparatus consisted of a glass tube (diameter = 2 cm, depth = 6 cm), the interior of which was lined from top to bottom with a section of filter paper that served as an egg deposition substrate. A total of 50 oviposition tubes were divided into five groups of 10 tubes each that were filled with one of the following solutions: (1) water (control), (2) RCC, (3) RCU, (4) GCC, and (5) GCU. Thirty minutes after transfer into tubes, the solutions were gently discarded and the tubes were immediately covered with lids formed from a 1.5-mL Eppendorf tube (with the lower bottom cut out) affixed across the cover of the tube at the middle of which was an aperture. The 50 gravid females within the 50 individual tubes (10 tubes with water, 10 with RCC, 10 with RCU, 10 with GCC, and 10 with GCU) had continuous access to 10% sucrose solution through cotton wicks placed across the removed bottom of the Eppendorf tube. After 3 days of egg laying opportunity, the 50 females were removed from the tubes and placed back in the original cage. The lids of glass tubes were left half-open to allow eggs to dry and embryonate at the deposition sites (moistened substrates) under laboratory conditions, as reported elsewhere (Dieng et al. 2014). After a 3-day drying period, the 10 filter papers that were imbibed with water and held dried eggs were carefully removed from the glass tubes and kept in 250-mL plastic vessels. A water-saturated cotton wick was placed inside each vessel to avoid desiccation. Similar treatments were also performed for each of the 10 dried substrates of each of the four remaining groups (RCC-soaked substrates, RCU-soaked substrates, GCC-soaked substrates, and GCU-soaked substrates). The same procedures and treatments as described above for *Ae. albopictus* were also performed for *Ae. aegypti*.

Coffee extracts and larval eclosion

Dried eggs of *Ae albopictus* and *Ae. aegypti* obtained from water-, RCC-, RCU-, GCC-, and GCU-soaked substrates were flooded twice to examine whether coffee affects the viability of embryos by preventing larval eclosion (Table 2). The eggs were placed in 250-mL plastic vessels containing 50 mL of tap water supplemented with 0.003 g of yeast. Twenty-four hours after submersion, egg hatching was checked by counting and collecting larvae. The hatching media were discarded. Unhatched eggs were permitted to dry inside the hatching vessels under conditions similar to those described earlier. After 3 days, they were reflooded and hatching responses were monitored the next day. The collected first instar larvae were used for the next experiment.

Coffee extracts and adult lifespan

To determine whether embryonic development in the presence of coffee affects adult longevity, *Ae. albopictus* larvae that hatched from eggs that matured on the different substrates were reared at a density of 50 in 500 mL of dechlorinated water, RCC, RCU, GCC, or GCU in metallic containers (diameter = 12 cm, depth = 2 cm). Larvae were given food (a 2:1:1:1 mixture of dog biscuits, beef liver, yeast, and milk powder) in suspension form as described above for producing the experimental subjects, but daily amounts were halved (1.5 mL on the day of hatching, and 3 mL on post-hatching days 3, 5, 7, and 9). Pupae were placed singly into 1.5-mL Eppendorf tubes containing 0.5 mL of tap water. Upon emergence, 10 adult males were gathered in one cage labeled "M" and 10 females in another cage labeled "F." The cages for water treatment-derived males and females

Table 2 Mean (±SE) numbers of eggs used in hatching experiments

Species	Substrate-soaking medium	Number of substrate replicates	Mean (±SE) numbers of eggs	Range
Ae. albopictus	Water	10	22.00 ± 0.0	22
	RCC	10	24.10 ± 2.54	15–43
	RCU	10	22.00 ± 1.14	17–30
	GCC	10	25.60 ± 1.55	21–37
	GCU	10	26.20 ± 0.89	20–29
Ae. aegypti	Water	10	25.20 ± 0.85	21–30
	RCC	10	25.60 ± 1.96	20–39
	RCU	10	22.30 ± 0.61	20–25
	GCC	10	23.50 ± 0.77	20–29
	GCU	10		

were designated as Mwater and Fwater, respectively. The cages of adults derived from the other egg treatments were designated as MRCC and FRCC, MRCU and FRCU, MGCC and FGCC, and MGCU and FGCU, respectively. Ten *Ae. aegypti* adults of both sexes were produced and divided into cages as described above. All five categories of adults (both sexes and both species) had continuous access to 10% sucrose solution and were maintained under controlled environmental conditions (29 ± 3.0 °C, relative humidity 75 ± 1%, and photoperiod 13 h light/10 h dark). Daily, the 20 adult populations (10 for each species) were surveyed and the number and sex of dead individuals were noted.

Data collection and statistical analysis

In the larval eclosion study, the total numbers of eggs that hatched after the first and second flooding events were summed and the resulting totals were used to compute egg hatching rates as the number of hatched eggs divided by the total number of eggs (unhatched + hatched) flooded × 100. In the longevity study, adult lifespan was expressed as the number of days between larval eclosion and adult death as described previously (Dieng et al. 2014). The differences in larval eclosion responses and lifespan between the different embryo development environments were analyzed by analysis of variance (ANOVA) using the Systat v.11 statistical software package (Systat Software Inc., Systat 11 data 2004). Tukey's post hoc test was used to examine the significance of differences between two given embryo maturation environments with regard to egg hatching and lifespan responses. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

Results

Egg hatching responses

In *Ae. albopictus*, egg hatching responses varied significantly with oviposition substrate medium ($F = 30.088$; $df = 4$; $P < 0.001$). The mean hatching rate of eggs that developed on water (99.54 ± 0.45%) was consistently greater than that of eggs laid on RCC-soaked substrates (28.10 ± 6.47%) [*Matrix pairwise mean differences* (*MPMD*) = − 71.445; $P < 0.001$]. This latter hatching rate was significantly lower than that of eggs maintained in the RCU-moistened environment (69.60 ± 7.43%; *MPMD* = 41.506; $P < 0.001$), which in turn was appreciably higher than that of eggs matured in the GCC environment (47.93 ± 5.04%; *MPMD* = − 21.676; $P = 0.040$). This latter mean larval eclosion rate was substantially lower than that of eggs matured on substrates soaked with GCU (85.53 ± 3.68%; *MPMD* = 37.609; $P < 0.001$) (Fig. 1a).

In *Ae. aegypti*, maturation of eggs in the presence of coffee extracts consistently affected hatching success ($F = 11.353$; $df = 4$; $P < 0.001$). The larval eclosion response was significantly higher in the water-exposed eggs (97.22 ± 1.39%) than in those exposed to coffee extracts. *Ae. aegypti* eggs maintained on RCC substrates exhibited a lower hatching success rate (49.77 ± 7.56%) than those that developed in the presence of RCU (87.45 ± 2.11%; *MPMD* = 37.677; $P < 0.001$), which was lower than that of GCC-conditioned eggs (85.32 ± 4.62%; *MPMD* = –13.774; $P = 0.385$). This latter hatching rate was similar to that recorded for eggs exposed to GCU (*MPMD* = 11.642; $P = 0.553$) (Fig. 1b).

Comparison of hatching responses between RCC vs. GCC and RCU vs. GCU

In the crude extract treatments, *Ae. albopictus* hatching rates tended to be greater for eggs that were exposed to substrates wetted with RC than GC, but the difference was not statistically significant (*MPMD* = 19.830; $P = 0.072$). An identical pattern was seen for the eggs treated with used extracts, with exposure to the roasted form associated with a higher hatching rate than the fresh form (69.60 ± 7.43 and 85.53 ± 3.68%, respectively; *MPMD* = 15.933; $P = 0.215$).

Ae. aegypti eggs reared on RCC showed a greater hatching rate than those that matured on GCC substrates

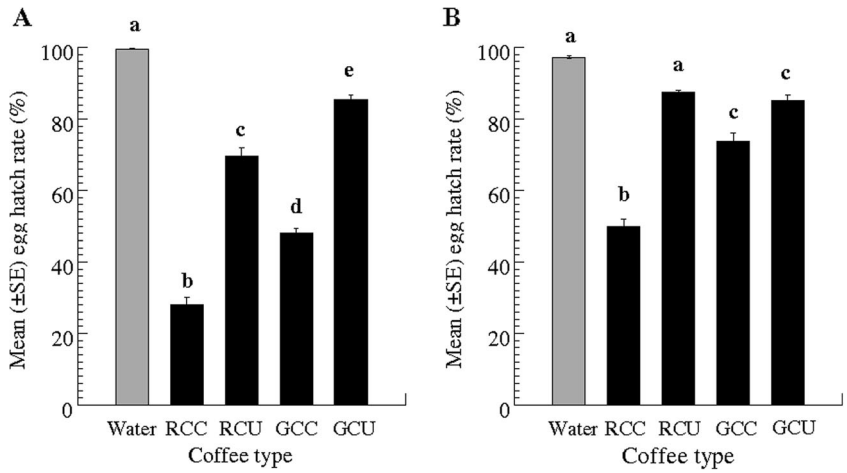

Fig. 1 Mean hatch rates (mean ± SE) of eggs *Ae. albopictus* (**a**) and *Ae. aegypti* (**b**) that were maintained on substrates moistened with water (control) and different coffee extracts. *Bars with the same letter* do not show a significant difference ($P < 0.05$) based on Tukey statistic for means comparison

(*MPMD* = 23.903; $P = 0.024$). There was no significant difference in hatching rates of *Ae. aegypti* eggs maintained on RCU and GCU crude extracts (*MPMD* = –2.132; $P = 0.999$).

Comparison of hatching responses between RCC vs. RCU and GCC vs. GCU

Among the different coffee treatments, egg hatching success in *Ae. albopictus* was lower in the case of crude forms (RCC and GCC) than on oviposition substrates moistened with the two used coffee extracts (RCU and GCU). A similar pattern of larval eclosion was also observed for *Ae. aegypti*.

Female longevity responses

The mean number of days of life of *Ae. albopictus* females that developed in the water-moistened milieu (43.30 ± 4.07 days) was higher than that of RCU treatment-derived females (41.60 ± 1.22 days), which in turn was lower than those in the RCC (40.60 ± 2.32 days) and GCC (39.80 ± 3.93 days) treatment groups. However, the differences between these four mean lifespans were not significant ($F = 0.227$; $df = 3$; $P = 0.877$) (Fig. 2a).

There were no significant differences in *Ae. aegypti* female longevity between the different embryo development environments (water, RCU, RCC, and GCC, DF = 3; $F = 0.549$; $df = 3$; $P = 0.652$). However, embryos matured on water-moistened oviposition substrate (50.60 ± 2.99 days) tended to show longer adult lifespan compared to those on substrates soaked with RCU (44.50 ± 3.89 days), RCC (43.50 ± 4.66 days), and GCC (45.30 ± 5.22 days) (Fig. 2a).

Male longevity responses

The lifespan of *Ae. albopictus* males did not vary significantly with egg development environment ($F = 2.035$; $df = 3$; $P = 0.126$). However, males derived from eggs exposed to

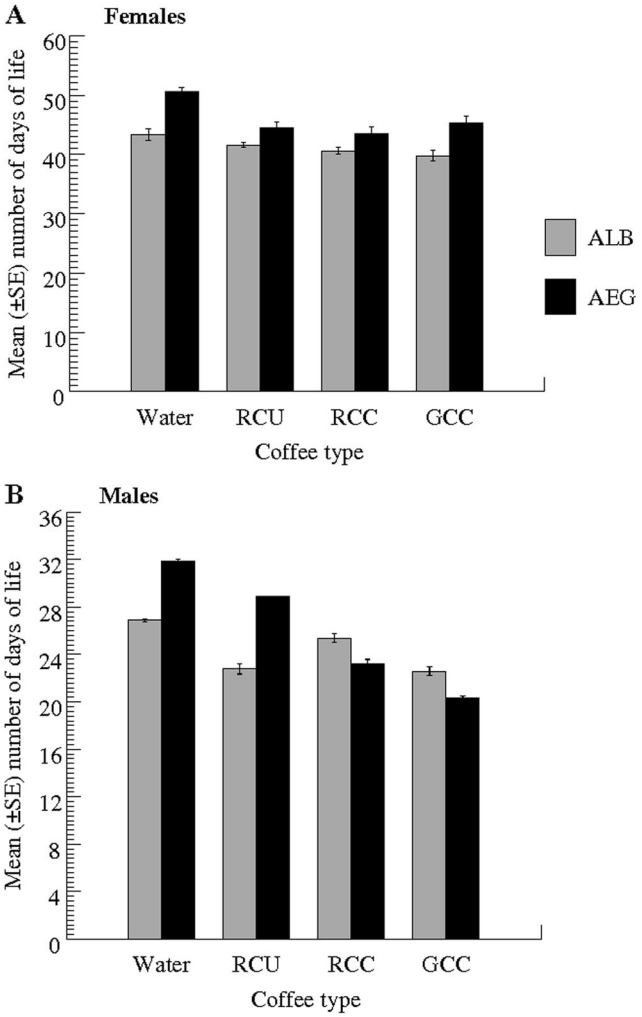

Fig. 2 Mean life spans (±SE) of *Ae. albopictus* and *Ae. aegypti* females (**a**) and males (**b**) derived from larvae that, in turn, derived from eggs that matured in a water moistened and different coffee extract-moistened environments

water during development tended to live longer compared to those matured on oviposition substrates moistened with coffee extracts (Fig. 2b).

The longevity of *Ae. aegypti* males varied significantly according to egg maturation environment ($F = 28.844$; $df = 3$; $P < 0.001$). The mean lifespan of males that matured as eggs on oviposition substrates submerged in water (31.90 ± 0.58 days) was similar to that of males derived from eggs that were kept in the RCU milieu (28.90 ± 0.10 days; $MPMD = -3.000$; $P = 0.154$), which in turn was consistently higher than that of males from the RCC treatment group (23.20 ± 1.64 days; $MPMD = -5.700$; $P = 0.001$). The longevity of RCC-derived males was shorter than that of their counterparts maintained in the GCC environment (20.30 ± 0.89 days), but the difference was not significant ($MPMD = -2.900$; $P = 0.177$) (Fig. 2b).

Comparison of longevity in roasted extract treatment groups (RCC and RCU)

With regard to roasted extract treatment, *Ae. albopictus* females that developed on crude extract during embryonic life and their RCU-derived counterparts had similar lifespans (41.60 ± 1.22 vs. 40.60 ± 2.32 days, respectively). Among RC-derived *Ae. albopictus* males, those derived from RCC treatment tended to live longer than RCU-derived males (24.40 ± 1.58 vs. 22.80 ± 1.84 days, respectively). There were no significant differences in lifespan of *Ae. aegypti* females raised on roasted extracts as eggs between those treated with RCC and their exposed to RCU medium (43.50 ± 4.66 vs. 44.50 ± 3.89 days, respectively). *Ae. aegypti* males from the RC treatment group that matured on RCC substrates had a shorter lifespan than those from the RCU treatment group (23.20 ± 1.64 vs. 28.90 ± 0.10 days, respectively).

Comparison of longevity in crude extract treatment groups (RCC and GCC)

With regard to roasted extract treatment of *Ae. albopictus*, RCC-derived females and their GCC-derived counterparts had similar ages (40.60 ± 2.32 vs. 39.80 ± 3.93 days, respectively). An equivalent pattern of discrepancy in lifespan was also observed in males (25.40 ± 1.58 vs. 22.60 ± 1.49 days, respectively). The lifespans were similar between RCC- and GCC-derived *Ae. aegypti* females (43.50 ± 4.66 vs. 45.30 ± 5.22 days, respectively) and between the corresponding males (23.20 ± 1.64 vs. 20.30 ± 0.89 days, respectively).

Discussion

Moderate levels of caffeine from coffee have no negative effects on birth success in humans (Browne et al. 2011), and coffee consumption is associated with low risk of death (Freedman et al. 2012). Here, we found that exposure of embryos during maturation limits larval eclosion and reduces adult lifespan of dengue mosquitoes. In control treatment (water), egg hatching rates were very high for both *Ae. albopictus* and *Ae. aegypti*. However, larval eclosion success rates were markedly reduced when maturation proceeded on coffee-moistened substrates. In addition, both males and females of the two species that matured as eggs in environments containing coffee tended to have shorter lifespans than their counterparts that developed in a water milieu. There have been a number of previous studies regarding the impacts of coffee or its components on insect pests. However, most of these studies focused on the effects of caffeine (Derraik and Stanley 2005; Laranja et al. 2006), which needs to be purified from coffee beans, a process that can be costly. In addressing this issue, Derraik and Stanley (2005) suggested that although caffeine appears to be a valuable mosquito larvicide, it would not be accessible to the general public and therefore not feasible for mosquito control programs. Some studies utilized spent coffee grounds (Laranja et al. 2003; Derraik and Stanley 2005), but most of these investigations were performed on larvae and did not examine eggs or adults. These two latter stages are central to dengue transmission because the virus can be transmitted both vertically and horizontally (Thenmozhi et al. 2007). The World Health Organization has advocated that the egg stage should be taken into account in containment strategies to address the problems of dengue vector control (Schofield 2000).

The present study examined whether various forms of coffee extracts could block mosquito development at the egg stage. Maturation in the presence of coffee resulted in hatching rates ranging from 28 to 85% in *Ae. albopictus* and from 49 to 85% in *Ae. aegypti*. In contrast, larval eclosion responses were almost optimal for *Ae. albopictus* and *Ae. aegypti* eggs raised on water-saturated substrates. In *Aedes* mosquitoes, including those studied here, successful embryo development and viability (Strickman 1980; Dieng et al. 2006) as well as hatching success require adequate water absorption (Saifur et al. 2010) via osmotic driving forces (Valencia et al. 1996). Immersion of *Anopheles* spp. eggs in 0.75 M sugar solution for 90 min resulted in marked shrinkage (Valencia et al. 1996). Rosay (1959) reported that *Ae. nigromaculis* eggs reared in a water environment increased in size, whereas those maintained in an oil milieu remained almost unchanged. Eggs of both *Ae. albopictus* and *Ae. aegypti* maintained under higher moisture conditions showed increased viability (Dieng et al. 2006; Hardwood and Horsfall 1956). Cigarette butt (CB) extract was shown previously to inhibit larval eclosion in *Ae. albopictus*, which was credited to the low levels of dissolved oxygen due to the antibacterial effects of some CB leachates (Dieng et al. 2011). Bacteria also promote egg hatching (Ponnusamy et al. 2011). Recently, Satho et al. (2015) investigated the effects of exposing *Ae. albopictus* eggs to different coffee

concentrations and reported significant decreases in larval eclosion rates when progressing from maturation on substrates imbibed with a high coffee extract to those imbibed with a mild coffee concentration to water-saturated substrates (almost 100%). They suggested that the increased ionic balance in the coffee solutions examined prevented sufficient uptake of water by the developing embryos. They also reported that the reduced hatching rates among coffee-treated eggs was due to the levels of DOPA decarboxylase activity, an enzyme that controls the darkening process of newly laid eggs (Li et al. 1996). In the present study, freshly laid eggs were allowed to develop for 3 days on their respective substrates (water, RCC, RCU, GCC, and GCU). Even under conditions where hatching responses were the same for all eggs, larval eclosion success in the water-exposed group was almost 100%, while eggs that developed on substrates saturated with coffee extracts showed considerable rates of hatching failure. Taking the above studies into account, the observed reduction of hatching could be explained by a failure to take up a sufficient amount of water for successful embryonation. It is possible that some coffee components exert inhibitory effects on bacteria present in the egg shell, which may have distorted the normal pattern of dissolved oxygen depletion. Low levels of phenoloxidase and related enzyme activities in coffee-exposed eggs may also explain the reduced hatching responses, as green coffee beans or components, such as caffeine, can have strong inhibitory effects on enzyme activities (Shimoda et al. 2006; Laranja et al. 2003).

Both roasted and crude coffee treatments affected *Ae. albopictus* and *Ae. aegypti* egg hatching success rates. However, larval eclosion rates from roasted coffee-treated eggs tended to be lower than those of eggs treated with natural coffee extract (green coffee). Specifically, hatching success rate following embryonic development on RCC was far lower than that following GCC exposure, suggesting that the RCC medium contained higher levels of agents that inhibited embryonic development and/or larval eclosion. The discrepancies in chemistry between roasted and fresh coffees have been well characterized. Coffee contains more than 1000 chemicals (Clarke 2013). Green coffee beans contain many compounds, such as alkaloids, caffeine and trigonelline, proteins, amino acids, carbohydrates, chlorogenic acids, chain fatty acids, aldehydes, and nitrogen-containing aromatic molecules (Clifford and Kazi 1987; Farah 2012). Roasting is a heating process in which the beans undergo physical and chemical changes (Franca et al. 2005). For example, whereas caffeine survives the roasting process (Wang 2012), 85% of trigonelline in green coffee beans is degraded to nicotinic acid at 230 °C (Poisson 1979). Chlorogenic acids, the most abundant polyphenols in coffee (Tapiero et al. 2002), are significantly affected by roasting (Gawlik-Dziki et al. 2014). Up to 54, 60, and 100% of chlorogenic acids are destroyed in light, medium, and dark roasted beans, respectively (Clifford 1979). Roasting markedly increases the chemical complexity of coffee. Unroasted green coffee beans contain roughly 250 different volatile molecular species (Illy 2002), and around 950 new compounds are generated after roasting (Farah 2012). For example, the degradation of some chlorogenic acids produces free phenolic acids, chlorogenic acid, and lactones or antioxidant high-molecular-weight products known as melanoidins (Davies 2011), which in turn includes within their composition many different low-molecular-weight polyphenols (Rufián-Henares and Morales 2007). Other factors influencing the chemical composition of coffee include brewing and extraction time (Farah 2012). Despite these potential losses, brewed coffee contains large numbers and varieties of chemicals (Farah 2012), including quinic acid, 5-hydroxymethylfurfural, methyl furan, furfuryl mercaptan, trigonelline, chlorogenic acid, caffeic acid, citric acid, malic acid, lactic acid, pyruvic acid, acetic acid, pyrazine, thiazole, quinolone, phenyl pyridine, caffeine, and ketones (McCamey et al. 1990) and diterpenes (Sridevi et al. 2011).

In the present study, we used four different coffee brews (RCC and RCU from RC and GCC and GCU from GC) using the same amount of coffee and soaking period. Therefore, differential chemistries due to differences in bean type or extraction time were unlikely. With regard to the studies mentioned above, it is likely that the amounts of chemicals were greater in RCC than GCC, and it is therefore likely that these crude extracts have greater chemical resources than used extracts (RCU and GCU). Caffeine has been reported to retard growth in some beetles (Hewavitharanage et al. 1999) and moths (Sehgal et al. 1977) by delaying DNA synthesis and inhibits enzymes in the nervous systems of herbivorous insects, causing reproductive deficits (Helmenstine 2014). Other deleterious effects of caffeine on insects have been reported. Caffeine alters insect esterase enzymes, known to be crucial for basic physiological processes (Laranja et al. 2003), alters the webbing ability of spiders (Foelix 2010), and kills some insects (Nathanson 1984; Wink 1992; Derraik and Stanley 2005). Coffee prevents egg development in flies (Itoyama and Bicudo 1997) and reduces the reproductive potential of mosquitoes (Laranja et al. 2003). Trigonelline, an alkaloid derived from methylation of the nitrogen atom in nicotinic acid, has been shown to induce birth defects in some animals (Brunton et al. 2005). Some diterpenes are toxic to insects (Céspedes et al. 2013) and fish embryos (Rakotobe et al. 2010). Chlorogenic acid acts as a chemical defense against herbivores (Leiss et al. 2009) and exhibits antibacterial properties (Lizzi et al. 1995). Quinic acid causes developmental abnormalities in fish (Subedi et al. 2014). Based on these reports, it is tempting to suggest that the eggs placed on RCC-imbibed substrates took up more toxicants than those exposed to other media, some of which may have jointly or singly exerted detrimental effects that resulted in unsuccessful embryonation. Such adverse conditions were likely less pronounced among eggs exposed to either RCU or GCU. It is

also plausible that the observed discrepancies in larval eclosion rates between RCC exposure and the other coffee treatments were due to lower levels of DOPA decarboxylase or dissolved oxygen-consuming bacteria in RCU-, GCC-, and GCU-treated eggs. Consistent with this suggestion, roasted coffee has been shown to have increased activities against a broad range of bacteria (Daglia et al. 1994; Rufián-Henares and Morales 2008a) due to a membrane-damaging activity (Rufián-Henares and Morales 2008b) related with their metal-chelating activity (Rufián-Henares and Morales 2009).

Adults derived from the coffee treatment groups tended to have shorter lifespans than water-derived adults, and among the eggs exposed to different coffee treatments, the lifespans of both males and females tended to be shorter in crude extract treatment groups, particularly RCC. In aquatic organisms with life cycles consisting of juvenile and adult forms, conditions prevailing during one life stage can affect subsequent life stages and generations (Green and McCormick 2005). Such effects are more pronounced in the case of environmental stresses from pollutants (Parsons 1990). Mosquitoes have both aquatic and terrestrial phases. *Aedes* lay eggs on the water surface and spend the larval and pupal stages in water (Clements 1999; 2000). Larvae feed by filtering the water (Yee et al. 2004), which can enter and leave the embryos via osmosis (Jacobs et al. 2013). The pharate larva inside an *Aedes* egg (Clements 1992) is sensitive to environmental stressors. Using three insect growth regulators and eggs from three *Aedes* species, Suman et al. (2013) showed that embryo responses to chemical stressors are dependent on the mode of action, type, and concentration of agent; the chitin synthesis inhibitor, diflubenzuron, altered cuticle chitinization, which resulted in abnormal eggshell hardening and aberrant hatching; the juvenile hormone analog, pyriproxyfen, and the ecdysone agonist, azadirachtin, disrupted hormonal activities. Stressors such as fungal biopesticides can penetrate the cuticular barrier of *Ae. aegypti* eggs (Luz et al. 2008), suggesting potential sublethal responses in the embryo. As the adult lifespan has crucial effects on dengue transmission and vectorial capacity (Costanzo et al. 2015), and with respect to insecticide resistance development (Chareonviriyaphap et al. 2013), many studies have shifted to examining the sublethal effects on adult dengue vectors. Most of these studies exposed larvae to insecticidal products. For example, Sawby et al. (1992) and da Silva et al. (2009) exposed *Ae. aegypti* larvae to sublethal concentrations of either methoprene or diflubenzuron, and the results indicated appreciable decreases in adult lifespan. Although the eggs of dengue vectors can lead to the spread of the disease (Thenmozhi et al. 2007), unlike larvae, there have been few investigations regarding the effects of chemical stress on adult fitness traits, such as lifespan (Perez and Noriega 2014; Ezeakacha 2015). The indirect effects of cigarette butts (CBs) on adult lifespan of *Ae. aegypti* were recently investigated by exposing newly laid eggs on substrates imbibed with CB extracts and by allowing hatched larvae to develop in CB media (Dieng et al. 2014). Significant reductions were observed in the lifespans of adults of both sexes exposed to CBs, and it was suggested that leachates from CBs hindered feeding activities thus resulting in insufficient nutrient storage. This suggestion was consistent with an earlier study on *Ae. aegypti* indicating reduced larval feeding activity in the presence of *Bacillus thuringiensis*, which was considered to be due to feeding deterrence activity of its toxins (Aly et al. 1988). In a related study, Lu et al. (2013) reported similar observations following exposure of developing *Ae. aegypti* larvae to fraxinellone, and it was suggested that the insecticide deterred feeding due to the presence of alkaloids, phenolic, and terpenic compounds. Studies on coffee or its components on insects have demonstrated detrimental effects on feeding activities and nutrient assimilation. For example, caffeine has been reported to inhibit food consumption in flies and beetles (Araque et al. 2007), as well as lepidopterans (Nathanson 1984). Chlorogenic acid is known to lower the bioavailability of amino acids and digestibility of dietary proteins by binding to them in its chlorogenoquinonic form. In our longevity study, newly hatched larvae obtained from the hatching experiments were reared at a density of 50 in 500 mL and provided the same amount of food. Males and females from all treatment groups were separately pooled in cages, and all had constant access to 10% sucrose solution. It is well established that *Aedes* larvae that accumulate sufficient nutrients emerge with large bodies (Sumanochitrapon et al. 1998). Size at emergence potentially has crucial effects on adult fitness. Indeed, growing a large body has a positive effect on fitness in both sexes (Schneider et al. 2004). Large-bodied *Ae. aegypti* males (Maciel de Freitas et al. 2007) and females (Reiskind and Lounibos 2009) live longer than small males because they have greater teneral reserves (Briegel 1990a), and *Ae. albopictus* is expected to show a similar pattern (Suzuki et al. 1993; Dieng et al. 2010). A recent study showed that exposure of these two species to a bioinsecticide during larval life resulted in decreased body size and longevity of both sexes (Kamiabi et al. 2013). In the present study, the presence of coffee during embryo maturation was associated with decreased adult lifespans in both species examined. Although we did not assess larval nutrient levels and adult biomasses, the reduced lifespan (at least numerically) in adults derived from coffee-treated eggs could be explained by the presence of low teneral nutrient reserves at emergence. Embryos within eggs maintained on coffee-contaminated substrates presumably incorporated some chemicals that were carried over on hatching. It is possible that contaminated larvae did not amass and/or assimilate sufficient nutrients simply because some incorporated coffee chemicals acted as feeding deterrents and/or interfered with absorption, resulting in small body size on emergence. For example, it is known that coffee melanoidins

have strong metal-chelating properties (Rufián-Henares and Morales 2009) responsible of their antimicrobial activity (Rufián-Henares and Morales 2008b). Therefore, it is plausible that coffee melanoidins could diminish the absorbance of important minerals by mosquito larvae, resulting in a poor nutritional status and decreased weight. These effects on feeding and assimilation were more marked in *Ae. aegypti* males derived from eggs that matured in the crude extract environments (RCC and GCC). In support of these suggestions, it has been reported that alkaloids, such as caffeine, deter feeding (Nathanson 1984; Araque et al. 2007) and that phenolic compounds, such as chlorogenic acid, reduce amino acid digestion in many insects (Jassbi 2003; Mallikarjuna et al. 2004).

Note that in both RCU and RCC treatments, *Ae. albopictus* females tended to die earlier than their *Ae. aegypti* counterparts, whereas in males, *Ae. aegypti* tended to die sooner. Based on previous reports, it is likely that parental larvae of *Ae. albopictus* females and those of *Ae. aegypti* males had weaker ability to ingest or assimilate nutrients in the presence of coffee toxicants and thus had reduced nutritional reserves as compared to *Ae. aegypti*. Therefore, it is likely that the larvae of *Ae. albopictus* females and *Ae. aegypti* males had better capacities to avoid or overcome the potential anti-feeding and anti-nutrient assimilation effects of chemicals present in coffee.

Conclusions

RCU altered hatching success and longevity (male) of both *Ae. albopictus* and *Ae. aegypti*, suggesting that the extract likely contains agents that aversely affect embryo development and larval eclosion. The observation that fresh and used coffee solutions can prevent larval eclosion and shorten the lifespan of dengue vectors would be auxiliary advantages to vector control programs. These attributes can reduce the total population size and number of individual mosquitoes capable of transmitting the virus. The structural and evolutionary chemical diversity of coffee (Farah 2012; Farah and Donangelo 2006; Clarke 2013) are likely to impose variations in mechanisms of action, a primary problem faced by vector control methodologies. In addition to providing insight into the potential of coffee extracts to assist in dengue vector control, this study suggests the possibility of developing potent, low-cost, and biorational coffee-based mosquito control strategies. This approach is likely to be successful in both modern societies where coffee waste is ubiquitous (Biocontrol Beat 2009) and in coffee-producing countries where huge amounts of pulp and effluent are discarded into rivers and lakes (Leibrock 2014). Reusing such waste in vector control could be a reasonable solution to the concerns associated with such pollution. The aqueous extract of coffee has already been reported as an attractive signal to ovipositing dengue mosquito females at a given concentration (Satho et al. 2015). Such an attribute coupled with the currently observed embryocidal activity suggest that the water extract of coffee could be used for integrated vector control, thereby reducing environmental repercussions often associated with the use of synthetic insecticides.

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