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Attractiveness of bioinsecticides caffeine and used coffee grounds in the choice of oviposition site by *Aedes aegypti* (Diptera: Culicidae)

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Abstract
Data in the literature have shown that treatment with caffeine (CAF) blocks development and causes death of *Aedes aegypti* (*Ae. aegypti*), in the larval stage. Since adults are not produced, CAF was considered a potential bioinsecticide, useful for controlling these mosquitoes that transmit human viral diseases. Similar results were obtained in tests with used coffee grounds (UCG). Considering the importance of the oviposition site in the reproductive success, in the present study, two experiments were carried out aiming to verify if CAF aqueous solution and UCG suspension might interfere in the choice of the oviposition site by *Ae. Aegypti*, competing with water, the attractive medium in their normal breeding sites. Four glasses were put into a cage, two containing CAF solutions in different concentrations, one containing UCG and other water. The oviposition behavior was verified by counting the number of eggs. CAF solutions showed a number of eggs greater than that in the other mediums. During the experiments, many mosquitoes died immersed in the mediums, being their number higher in UCG. These observations are discussed, reinforcing the possibility of using CAF and UCG as auxiliaries in the control of *Ae. Aegypti*.

Keywords: caffeine, used coffee grounds, oviposition, *Aedes aegypti.*

1. Introduction
Ae. aegypti is responsible for transmission of arbovirus that cause several serious human diseases, including dengue, dengue hemorrhagic fever (DHF), yellow fever, chikungunya and Zika. In many countries located in the tropical and subtropical regions, the great density of these mosquitoes has caused epidemics and high rate of mortality. A study published in 2012 estimated that 3,900 million people, in 128 countries, were at risk of infection with dengue viruses [1]. Since the extension of the affected areas has grown continuously, these numbers may also have increased. Unfortunately, it is possible to say that, presently, in the battle between mosquitoes and man the mosquitoes have proven to be the great winners.
Ae. aegypti is difficult to be controlled due to high productivity and other specific biological characteristics that are very advantageous for reproduction and survival. It is amazing what can serve as breeding sites for this mosquito: any container that can accumulate water, even in small quantities, such as bottle caps and small pieces of plastic or other material, inside or outside homes. Larvae have been found in unexpected places such as the water inside the steam iron, or the "channel" where the door of the shower box runs. It is impossible to make a complete list of the expected breeding sites because each country, each region and each house have peculiarities. So, people living in the affected regions are encouraged to perform the important task to eliminate or control the mosquito breeding sites. If well performed, this task can greatly reduce the mosquito populations size, consequently reducing the spread of the diseases that they transmit. In the places where this is not done as needed, the use of insecticides has been the most widely used method for this purpose. This is not adequate although necessary in some cases. It is well known since a long time that insecticides are toxic to humans and the environment [2]. Additionally, insecticides have caused selection for resistance in the mosquitoes, forcing the use of higher doses and stronger formulations to be effective in the control programs [3, 4].
Efforts have been done since a long time ago to develop a vaccine for dengue, still without an effective success. More recently, tests are starting to be made with mosquitos genetically modified or infected for producing sterile males, which are released in great amounts, aiming to decimate mosquito populations in delimited areas.

Many alternative control methods using extracts of plants and seeds were tested and are described in the literature [5]. The use of most of them would involve collecting in the woods or planting several different vegetable species followed by the extraction in laboratory. One of the alternative methods described in the literature involves using, in breeding sites, solutions of caffeine (CAF), a substance that is already available for purchase in the market. Presently, CAF is normally used in beverages, medicines and cosmetics. Pure CAF (1,3,7-trimethylxanthine) is a white crystalline and odourless powder found in the composition of many plants, among which the most important sources are coffee (*Coffea* spp.), tea (*Camellia sinensis*), mate (*Ilex paraguariensis*), guarana (*Paullinia cupana*), cola nuts (*Cola vera*), and cocoa (*Theobroma cacao*) [6]. CAF can also be synthesized in laboratory [7]. It is considered an adenosine antagonist because the similarity between the molecular structures of both substances allows CAF to bind to the same receptor sites as adenosine [8].

In *Drosophila* species, different CAF concentrations had shown significant decrease in the progeny number. This effect was dose dependent and affected also other traits such as development time, mean longevity, oviposition and mitosis rates [9, 10, 11, 12]. These results in *Drosophila* species suggested testing the effects of CAF in *Ae. aegypti*. Depending on the results, this substance could be suitable for the control of this mosquito, in breeding sites that couldn't be eliminated. The results showed that this substance could block the development of these mosquitoes, killing them before reaching the adult phase [13, 14, 15]. Preventing the adult production is an important feature of a substance intended to be used in control programs of *Ae. aegypti* because the disease viruses are transmitted among persons by the bite of infected adult females (*Aedes* females need to ingest blood for completing egg maturation). Once infected, the mosquito female will remain infected during their life that lasts about four weeks and will transmit the viruses in each of the several blood meals they take [16].

Toxicological tests were also performed in *Ae. aegypti* with culture mediums containing used coffee grounds (UCG), which is the coffee powder that remains after preparing the beverage. UCG contain CAF remains and other components such as tannins and polyphenols that may cause toxicity [17, 18]. UCG provoked, in the mosquitoes, harmful effects similar to those caused by pure CAF [13; 19]. UCG and CAF affected the synthesis of esterase enzymes, which are located in brain, suggesting the possibility that they are involved with the control of molting hormones [13]. Other studies analyzed toxicity of UCG, confirming mortality, as for example for larvae of Culicidae *Ochlerotatus notoscriptus*, which is a vector of canine heartworm [20].

Considering that the choice of the oviposition site is a very important factor in the reproductive success of the mosquitoes, in the present study tests were performed to find out if CAF solution and UCG suspension could interfere in the mentioned choice by *Ae. aegypti*. If either of these culture mediums were more attractive or at least as attractive as water alone, the eggs developed in it would not surpass the larval stage, as shown in the studies using the two substances; this would help to reduce the population size of the mosquitoes, serving as an additional control method that would reinforce the value of these substances for *Aedes* control.

2. Material and Methods

Origin and maintenance of the mosquitoes in the laboratory

Aedes aegypti were collected in São José do Rio Preto - SP by technicians of the Superintendence of Endemics Control (SUCEN). In the laboratory, the mosquitoes were raised at room temperature, in cages (50.0cm width, 50.0cm height and 50.0cm length) having fine nylon screen in the walls and top, and presenting, in the anterior wall, an opening protected by fabric for handling inside the cage. The adults were fed with 0.08 g/mL sugar aqueous solution put in a narrow neck flask containing a partially submerged cotton wad so that the mosquitoes could suck the solution, which was renewed twice a week. As we mentioned since females need blood meals for the oocyte maturation, the mosquitoes were fed twice a week with mouse blood.

Glasses (7.0cm diameter X 7.50cm high) half filled with medium were put inside the cages for collecting the eggs. A wide ribbon of filter paper was partially immersed in the limit of the medium surface, encircling the entire interior of the glasses. The eggs were laid on this filter paper.

Experiments

Two experiments were carried out. In each of them, four glasses containing 200 mL of medium prepared for collecting the eggs were put into a raising cage. Inside the cage, the glasses were arranged in four positions, according to the drawing shown in Figure 1.

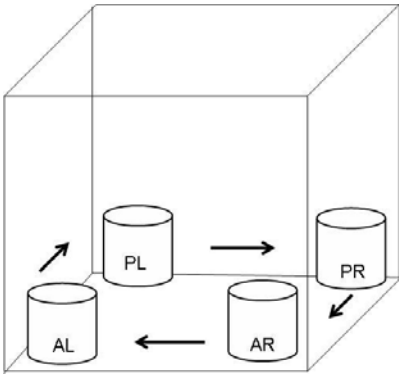

Fig 1: Drawing of the cage used in the experiments, showing the location of the four glasses with different mediums for *Aedes aegypti* oviposition. The arrows indicate the direction of the glasses rotation to occupy different positions inside the cage. AR=anterior right. AL=anterior left, PR=posterior right and PL=posterior left

In the first experiment, the mediums were CAF aqueous solutions at 0.5 and 1.0 mg/mL concentrations, UCG aqueous suspension at 150 mg/mL (equivalent to two full tablespoons) and water (control). In the second, the only difference was that CAF at 2.0 mg/mL was used instead of 1.0 mg/mL.

Twenty mosquito couples were introduced in the cages at the beginning of the experiments. Dead mosquitoes were replaced daily, resulting in the use of a total of 563 and 343 mosquitoes, in the experiments 1 and 2, respectively (Exp 1: 297 females and 266 males; Exp 2: 176 females and 167 males). New mediums were prepared when the females were fed with blood. On this occasion, the glasses with new mediums were rotated inside the cage from left to right to take up a new position relatively to the previous one. The aim was to avoid that the location of the glasses inside the cage could influence the attractiveness of the mosquitoes. The ribbons put inside the

glasses for oviposition were examined daily and were changed for news when they contained eggs. The ribbons removed were put to dry during 24 hours approximately and the eggs were counted with the aid of a magnifier lens.

Statistics

For statistical analysis of the tests, Graph Pad software from the computer program Prism 6.0 (2013) was used.

3. Results and Discussion

Counting the egg numbers

The experiments lasted 53 and 43 days, involving 22 and 29 egg counts in the four glasses containing different mediums, in the experiments 1 and 2, respectively (Table 1).

Table 1: Total number (TN) and percentage of eggs obtained in all counts, the mean number in each count and the standard deviation (sd) per kind of medium and experiment (EXP). The number of counts in which each medium showed the greater number of eggs for each position in the cage is also presented. The total numbers of counts in EXP 1 and EXP 2 were 22 and 29, respectively.

EXP	Mediums (mg/mL)	TN eggs (%)	Mean/count (sd)	Counts with grater OP per position				
				PL	PR	AL	AR	Totals
1	CAF 0.5	911 (39)	41.41 (55.83)	4	3	1	3	11
	CAF 1.0	667 (29)	30.32 (64.26)	1	2	2	2	7
	UCG 150	308 (13)	14.00 (18.36)	2	0	1	0	3
	Water	444 (19)	20.20 (36.42)	1	1	0	0	2
	Total	2330 (100)						
2	CAF 0.5	748 (27)	25.80 (36.80)	3	1	3	2	9
	CAF 2.0	960 (35)	33.10 (60.36)	1	2	4	4	11
	UCG 150	273 (10)	9.41 (25.91)	0	0	0	0	0
	Water	799 (28)	27.55 (31.56)	2	2	3	2	9
	Total	2780 (100)						

PL = Posterior left; PR = Posterior right; AL = Anterior left; AR = Anterior right; T = Total; N = Number; OP = Oviposition Preference.

As mentioned elsewhere, the glasses were rotate inside the cage when new mediums were used so that the different mediums occupied the different positions during the experiments. This concern was due to the knowledge that *Aedes* females are attracted more to darker than to lighter places or objects [21]. The rotation was an additional care since apparently the lighting inside the cage was homogeneous due to the size relatively small of the cage and to that the position of lights was central in the laboratory ceiling.

In both experiments, the oviposition rate showed significant difference among mediums (Exp. 1: $X^2 = 2275.2$, P=<0.0001; Exp 2: X^2=1980.2, P=<0.0001) and the oviposition rate in mediums containing CAF, independent of the concentration, was higher than that in UCG. In comparison with water, the results differed between experiments 1 and 2. In the experiment 1, the percentage oviposition in CAF 0.5 was more than twice that observed in water (51.27%), while in the other, percentage in CAF 0.5 was 1.068 smaller than that in water due to a great increase of egg number in the latter medium.

The sum of the number of eggs obtained in the two CAF concentrations, in each experiment, showed a percentage higher than 60% considering oviposition in all mediums (Exp 1=68.0%; Exp 2=62.0%), indicating that the mediums containing CAF were the most attractive for oviposition, while the mediums containing UCG were the less attractive (13.0% and 10.0%, in the Exps 1 and 2, respectively). The values in water were 19.0% and 28.0%, respectively. The difference of egg numbers between the two CAF concentrations was low (10.0% and 8.0%, in the experiments 1 and 2, respectively). Considering this finding and the fact that the difference between the experiments relative to the total percentage of eggs in the two CAF concentrations was also low (68.0%-62.0%=6.0%), it was inferred that the substance rather than the concentrations used influenced the choice of CAF as oviposition site.

The preferential choice of mediums containing CAF for oviposition may be interesting for control programs of these vectors. Presently, this control is based exclusively on the decrease of the mosquito population size since an efficient vaccine for dengue is not yet available (one that has about 60% efficiency, produced in France, is being tested). As mentioned else were, CAF blocks development of the mosquitos, killing them at the larval stages [13, 14, 15]. This effect is dose dependent, in general killing 100% of larvae at 1.0 mg/mL concentration. Thus, choosing culture media containing CAF for oviposition, the females are decreeing the death of the larvae that will develop from the eggs. The same is expected to occur to the larvae developed from eggs laid in mediums containing UCG; they will die as shown by results of previous studies [13, 19].

Adult mortality in the mediums

During the experiments, mosquitoes dead in the glasses containing the mediums were observed. Due to their relatively small numbers, for statistical analysis purpose the mosquitoes dead in each kind of medium, in the two experiments, were

summed. The comparison showed significant difference among mediums (X^2=11.210, P<0.0037). The counting of dead adults showed that more mosquitoes died in UCG (about 49.0% and 60.0% of the total died in the four mediums, in the experiments 1 and 2, respectively). In decreasing number was the sum of dead mosquitoes in the two CAF concentrations in each experiment (about 41.0% and 35.0% in the Exps 1 and 2, respectively). The lowest number was observed in water, which is the natural breeding medium of the mosquito (about 10% mortality in each Exp) (Table 2).

Table 2: Number of adults that died in each medium, in the total counts of the two experiments (Exps). CAF= caffeine. UCG= used coffee grounds, M= males and F= females.

Exps	CAF (mg/mL)									UCG (mg/ml)			Control		
	0.5			1.0			2.0			150			Water		
	M	F	T (%)	M	F	T (%)	M	F	T (%)	M	F	T (%)	M	F	T (%)
1	22	31	53 (19.93)	29	28	57 (21.43)		----		49	81	130 (48.87)	15	11	26 (9.77)
2	7	10	17 (9.77)		----		18	25	43 (24.71)	42	62	104 (59.77)	8	2	10 (5.75)

The dead mosquitoes in each medium were of both sexes. In the light of numbers, except in water of experiment 2, more females died than males. Mediums containing UCG, in addition to the greatest number of dead mosquitoes, showed the greatest difference between sexes in the number of dead mosquitoes, in both experiments: in the first, of total 130 dead mosquitos, 68.3% were females and in the second, of total 104, females were 59.6%.

Although normally males and females feed on the sugar solution present in a separate glass inside the cage, these observations show that they are also attracted to the other mediums, apparently for oviposition (females) and for feeding (males and maybe females too). The predominance of females may be expected, considering that they necessarily have to seek a medium for oviposition.

Considering data in the literature, UCG would be the best candidate to be the more attractive among the mediums used, due to the dark colour and the smell. It is known that vision and odors play an important role in the attraction of insect females for oviposition and host search. Black ovitraps have shown preference, competing with other traps light in color, while chemical variation has also shown to be effective in surveillance programs [21-24].

The mortality percentages in UCG may be indicative that it attracts mosquitoes more than expected from the number of eggs observed. An attempt to explain is that when the wings of the mosquitoes get wet in contact with the UCG suspension, it is difficult for them to go out of the medium due to its viscosity. If this happens to females, oviposition could be reduced or prevented, explaining the lower number of eggs in this medium.

Nevertheless, the present data show that in mediums containing UCG, in addition to the fact that100% emerged larvae die in concentrations of about 300mg/mL [13; 19], the attraction and death of high number of adults prevent males and females to contribute to the increase of the population size and consequently to the spread of disease virus. The same is true for mediums containing CAF, where besides the high number of adult deaths, the eggs developed are also "condemned" to death in the larval phase [13, 14, 15].

4. Conclusion

It is important to remember that unlike insecticides that are dangerous for human health (the insecticides normally used for *Ae. aegypti* control are organophosphorous and pyrethroids), CAF and UCG have taken part in human life since many centuries ago. There are records that tea was already consumed, in China, 4,700 years ago and coffee, in Etiopia, about 1,300 years ago [25, 26]. Currently, as mentioned, CAF is intensively used not only in beverages but also in medicines and cosmetic products, meaning that it is legally considered safe for normal human consumption. About the use of UCG in control programs, there is the additional advantage of being free of costs since, in the domestic environment it is normally discarded or used as fertilizer for plants [27, 28]. The findings of the present study will allow to characterize both mediums as promising to prepare adult traps, adding reasons to consider the two substances as potential helpful elements in the control of *Ae. aegypti*.

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6. References

1. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG *et al*. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 2012; doi:10.1371/journal.pntd.0001760.
2. http://www.toxicsaction.org/problems-and-solutions/pesticides the problem with pesticides. Toxics Action Center, (visited in July 13, 2015). 2012.
3. Dusfour I, Thalmensy V, Gaborit P, Issaly J, Carinci R, Girod R. Multiple insecticide resistance in *Aedes aegypti* (Diptera: Culicidae) populations compromises the effectiveness of dengue vector control in French Guiana. Mem Inst Oswaldo Cruz, Rio de Janeiro. 2011; 106:346-352.
4. Macoris MLG, Andrighetti MTM, Wanderley DMV, Ribolla PEM. Impact of insecticide resistance on the field control of Aedes aegypti in the State of São Paulo. Rev Soc Bras Med Trop. 2014; 47:573-578.
5. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. Indian J Med Res. 2012; 135:581-598.
6. Clifford MN, Ramirez-Martinez JR. Chlorogenic acids and purine alkaloids contents of mate (*Ilex*

paraguariensis) leaf and beverage. Food Chem. 1990; 35: 13-21.
7. González-Calderón D, González-Romero C, González-González CA, Fuentes-Benítes A. Synthesis of caffeine from theobromine: Bringing back an old experiment in a new setting. Educación Quimica. 2015; 26:9-12.
9. Higgins GA, Grzelak ME, Pond AJ, Cohen-Williams ME, Hodgson RA, Varty GB. The effect of caffeine to increase reaction time in the rat during a test of attention is mediated through antagonism of adenosine A2A receptors. Behav Brain Res. 2007; 18:32-42.
10. Itoyama MM, Bicudo HEMC. Effects of caffeine on fecundity, egg laying capacity, development time and longevity in Drosophila prosaltans. Rev Bras Genet. 1992; 15: 303-321.
11. Itoyama MM, Bicudo HEMC, Manzatto AJ. Effects of caffeine on mating frequency and pre-copulation and copulation durations in Drosophila prosaltans. Cytobios. 1995; 83: 245-248.
12. Itoyama MM, Bicudo HEMC, Cordeiro JA. Effects of caffeine on mitotic index in Drosophila prosaltans (Diptera). Rev Bras Genet. 1997; 20:655-658.
13. Itoyama MM, Bicudo HEMC, Manzatto AJ. Effect of the stannous chloride combined with caffeine on productivity of Drosophila prosaltans. Genet Mol Biol. 2000; 23:105-107.
14. Laranja AT, Manzatto AJ, Bicudo HEMC. Effects of caffeine and used coffee grounds on biological features of Aedes aegypti (Diptera, Culicidae) and their possible use in alternative control. Genet Mol Biol. 2003; 26:419-429.
15. Laranja AT, Manzato AJ, Bicudo HEMC. Caffeine effect on mortality and oviposition in successive generations of Aedes aegypti. J Publ Health. 2006; 40:1112-1117.
16. Guirado MM, Bicudo HEMC. Effect of caffeine on larval mortality of Aedes aegypti: Efficiency related to solution concentration and age. J Entomol Res. 2010; 34:11-21.
17. http://www.who.int/topics/dengue/en/ World Health Organization, Health Topics: Dengue. Updated May 2015, (visited August 2015).
18. Mussato SI, Machado EMS, Martins S, Teixeira JA. Production, Composition, and Application of Coffee and Its Industrial Residues. Food Bioprocess Technol. 2011; 4: 661-672.
19. Monente C, Ludwig IA, Irigoyen A, De Peña MP, Cid C. Assessment of Total (Free and Bound) Phenolic Compounds in Spent Coffee Extracts. J Agric Food Chem. 2015; 63:4327-4334.
20. Guirado MM, Bicudo HEMC. Effect of Used Coffee Grounds on Larval Mortality of *Aedes aegypti* L. (Diptera: Culicidae): Suspension Concentration and Age versus Efficacy. BioAssay. 2007; 2:1-7.
21. Derraik JGB, Slaney D. The toxicity of used coffee grounds to the larvae of *Ochlerotatus* (Finlaya) *notoscriptus* (Skuse) (Diptera: Culicidae). Annals of Med Entomol. 2005; 14:14-24.
22. Hoel DF, Obenauer PJ, Clark M, Smith R, Hughes TH, Larson RT, Diclaro JW, Allan SA. Efficacy of Ovitrap Colors and Patterns Forattracting *Aedes albopictus* at Suburban Field Sites in North-Central Florida. J Am Mosq Control Assoc. 2011; 27:245-251.
23. Bentley MD, Day JF. Chemical ecology and behavioral aspects of mosquito oviposition. Ann Rev Entomol. 1989; 34:401-421.
24. Zwiebel LJ, Takken W. Olfactory regulation of mosquito–host interactions. Insect Biochem Mol Biol. 2004; 34: 645–652. doi: 10.1016/j.ibmb.2004.03.017.
25. Afify A, Galizia CG. Chemosensory Cues for Mosquito Oviposition Site Selection. J Med Entomol. DOI: http://dx.doi.org/10.1093/jme/tju024. First published online: 5 February 2015.
26. Timson J. Caffeine. Mutat Res. 1977; 47:1-52.
27. Griffiths RR, Woodson PP. Caffeine physical dependence; a review of human and laboratory animal studies. Psychopharmacology; 1988; 94:437- 451.
28. Bravo J, Juániz I, Monente C, Caemmerer B, Kroh LW, De Peña MP, Cid C. Evaluation of Spent Coffee Obtained from the Most Common Coffeemakers as a Source of Hydrophilic Bioactive Compounds. J Agric Food Chem. 2012; 60:12565-12573.
29. http://www.sunset.com/garden/earth-friendly/starbucks-coffee-compost-test. Using Coffee Grounds in the Garden - Sunset (visited July 15, 2015).