Received: 06/X/2006
Accepted: 10/IV2007
Published: 31/V/2007
Available online at: www.bioassay.org.br/articles/2.5

CHEMICAL CONTROL

# Effect of Used Coffee Grounds on Larval Mortality of *Aedes aegypti* L. (Diptera: Culicidae): Suspension Concentration and Age *versus* Efficacy

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*BioAssay 2:5 (2007)*

Efeito da Borra de Café na Mortalidade Larval de *Aedes aegypti* L. (Diptera: Culicidae): Concentração e Idade da Suspensão *versus* Eficácia

**RESUMO –** Em estudo anterior, a borra do café afetou o desenvolvimento larval de *Aedes aegypti* L. No presente trabalho, foi analisada a duração do efeito na mortalidade larval, de suspensões aquosas de borra do café, nas concentrações 75, 150, 250 e 300 mg/ml. A mortalidade larval nos criadouros experimentais foi acompanhada diariamente: a concentração 300 mg/ml foi a mais eficiente, produzindo 100% de mortalidade até nove dias após o preparo da suspensão. Estes resultados foram observados tanto em experimentos nos quais as larvas permaneceram livres podendo fazer contato com o depósito de borra do café no fundo dos frascos e com o sobrenadante líquido, como também nos experimentos em que as larvas foram mantidas em uma peneira de tela fina imersa na parte líquida da suspensão. Embora a eliminação dos criadouros seja a melhor maneira de controlar o tamanho das populações de *A. aegypti*, os resultados deste estudo reforçam a validade de considerar a borra do café como um possível auxiliar no controle deste mosquito, principalmente em jardins. A borra do café tem a vantagem de ser livre de custo, pois é o pó deixado no coador e jogado fora depois que a bebida é preparada.

**PALAVRAS-CHAVE:** *Aedes aegypti*, controle alternativo, borra do café

**ABSTRACT -** In a previous study, used coffee ground affected the larval development of *Aedes aegypti.* In this work, we evaluated the duration of the effect on larval mortality of aqueous suspensions of used coffee ground at 75, 150, 250 and 300 mg/ml concentrations. The larval mortality was followed daily, in the experimental breeding sites; 300 mg/ml was the most efficient concentration, producing 100% of larval mortality until nine days after preparation. These results were observed in experiments in which the larvae remained free in the vials, making contact both the used coffee ground deposits and the supernatant liquid, and also in experiments in which the larvae were maintained in a sieve of fine screen immersed in the liquid part of the suspension. Thus, although the elimination of the breeding sites remains being the best way to control *Aedes aegypti* population size, the results obtained herein reinforces the validity of considering used coffee ground preparations as possible auxiliary in the alternative control of this mosquito, mainly in the gardens. Used coffee ground has the advantage of being free of cost, since it is the powder that is left after coffee has been filtered out to drink.

**KEYWORDS:** *Aedes aegypti*, alternative control, used coffee ground

The mosquito *Aedes aegypti* L. is presently considered the one that shows the greatest dispersion in urban areas of the world (Silva *et al.* 2004). In many of those regions, *A. aegypti* is a vector of virus that causes serious human diseases, such as dengue, dengue hemorrhagic fever and yellow fever. Several factors contribute for the high density of mosquito populations. Among them are the great amount of garbage produced presently by human activities, part of them potential breeding sites for the mosquito and the globalization of human activities allowing the transport of mosquitoes between regions and countries. The mosquito is controlled mainly by the use of insecticides, whose toxic effects for man and the environment are well known (Slosek 1986, Marzochi 1994, Chauhan *et al.* 2000, Tian *et al.* 2000). The situation is being

aggravated by the fact that the mosquitoes are developing insecticide-resistance, causing the frequent need for the application of higher doses of those substances, their improper use in mixtures and the substitution of one insecticide by another of stronger effects (Macoris *et al.* 2003, Sousa-Polezzi & Bicudo 2004). These facts make it highly desirable to find alternative ways to control the *Aedes* population size. Mainly products originate from plants have been described for this aim (Furtado *et al.* 2005; Promsiri *et al.* 2006).

Used coffee grounds (UCG), the powder that is left after coffee has been filtered out to drink was used in tests for mortality of *A. aegypti* (Laranja *et al.* 2003). UCG suspensions in appropriate concentrations blocked the development of the mosquito before reaching the adult phase, in which the virus transmission occurs through the bites of the female.

In the present study, experiments for increasing information referring to the use of UCG were carried out, looking at the possibility of its use as auxiliary in the alternative control of *A. aegypti*. More specifically, in this study the durability of the effect of UCG suspensions on larval mortality (LM) was analyzed, since this is an important aspect of any substance-candidate for the aforesaid purpose.

## Material and Methods

In this study, larvae and pupae of *A. aegypti* were collected two or three times a month in breeding sites (rain-filled tires, cans, etc.), in an urban area from São José do Rio Preto - SP, by technicians from SUCEN and brought to the Vector Laboratory, at the Department of Biology – UNESP / IBILCE, where they were used to originate the cultures. In the laboratory, the mosquitoes, in the above-mentioned stages were placed for development in glasses (tumblers) containing tap water, inside wood cages with fine nylon screen walls. Their progeny, in the larval sub-phases L3 and L4, recognized on the basis of size, were submitted to treatments with UCG.

The characteristics basically analyzed in the present study were the concentration and the age of the aqueous suspensions of UCG in number of days after their preparation. The treatments were carried out in two conditions: allowing the larvae to enter in contact with the UCG deposits in the bottom of the experimental glass or preventing them from doing so. The effect of the addition or not of macerated fish food to the experiments, for feeding larvae, was also analyzed.

The UCG suspensions involved the use of a mixture of different brands of coffee.

**First Experiment.** The larvae were placed directly into the glasses bearing 200 ml of the suspension. In this case they were free to make contact both with the UCG deposits in the glass bottom and with the supernatant liquid. The concentrations of dry UCG used were 75, 150, 225 and 300 mg/ml, corresponding approximately to one, two, three or four level tablespoons, respectively. Five glasses were prepared of each of the four concentrations and of the tap water used as control. Daily, 10 larvae were placed into five glasses, four of which contained a different concentration of the medium and the fifth the control, till all the replicates had been used. The glasses were covered with a fine mesh fabric fixed with a rubber band. The analysis was daily, approximately in the same schedule, computing the number of live and dead larvae and the range treatment time to death. When all the larvae in a glass had died or developed to the adult stage, it was reused for 10 new larvae. The analyses in this experiment involved suspensions aged up to 12 days, due to this reuse of the glasses. The age is the time elapsed since the suspension was prepared. Fish food was not added to the suspensions.

**Second Experiment.** The larvae were maintained in a sieve of fine screen immersed into the liquid part of the UCG suspension, restricting them from touching or going into the UGC deposit located at the bottom of the treatment glass. This experiment involved the same concentrations and the same number of replicates of the First Experiment. Two of the five replicates in each concentration (the one-day and four-day suspensions) received the addition of 0.01 g of food per 200 ml of suspension. The analysis was carried out as in the First Experiment, and suspensions aged up to 15 days were used.

**Statistical Analysis**

Statistical analysis involved the use of linear correlation between UCG suspension age and percentage larval mortality in 48h (Zar, 1999).

## Results and Discussion

**First Experiment.** The results of this experiment, which involved the use of larvae placed unrestrictedly in the medium, are set out in Table 1. The suspensions containing 75 mg/ml UCG, out of the nine tests, three, aged between zero (used immediately after preparation) and two days, produced 100% LM after one or two days' treatment, and six tests with media aged between three and 12 days produced from 70% to 90% LM, after five to 13 days. These six tests yielded a total of 12 adults.

An increase in the concentration of the suspension also increased the percentage of tests with 100% LM, and its 100% mortality efficacy lasted longer, extending to the age of nine days in the medium 300 mg/ml UCG. For older suspensions of this concentration, the treatment time in which 100% mortality was obtained was extended to nine days, while for younger suspensions (up to four days old) the treatment time for 100% LM was 24h.

For more details, Table 2 shows the total results obtained in the First Experiment, in which the larvae

were free in the medium, being the normal way to use the UCG.

Statistical analysis using data from this Table showed that the linear correlation coefficients of UCG suspension age and percentage larval mortality in 48h, for every concentration used, was inverse: for 75mg/ml concentration, R= -0.758 (Figure 1A); for 150 mg/ml, R= -0.854 (Figure 1B); for 225 mg/ml, R= -0.759 (Figure 1C); and for 300mg/ml, R= -0.876 (Figure 1D).

**Second Experiment.** Table 1 also shows that, in the experiments, in which the larvae were maintained in a sieve placed within the liquid part of the suspension, two of the six tests using the medium without the addition of food, with 75 mg/ml UCG, aged zero and three days, produced 100% LM after six and four days' treatment, respectively. A suspension with the same concentration, aged 15 days produced 100% LM in 19 days. The remaining three tests (aged two, seven and nine days) showed from 70% to 80% mortality and produced seven adults. In the two tests with the addition of food (aged one and four days), LM was 70% and 90% and the number of adults produced was four.

As in the First Experiment, in those tests carried out without the addition of food, as the medium concentration increased, the percentage of tests with 100% LM and the limit of the suspension age able to produce that rate also increased. The same was observed in the media with the addition of food. The two tests using the medium with the highest UCG concentration (300 mg/ml) and the addition of food produced 100% mortality. In the Second Experiment the effect of the suspension on the 100% mortality, in tests without addition of food, lasted longer.

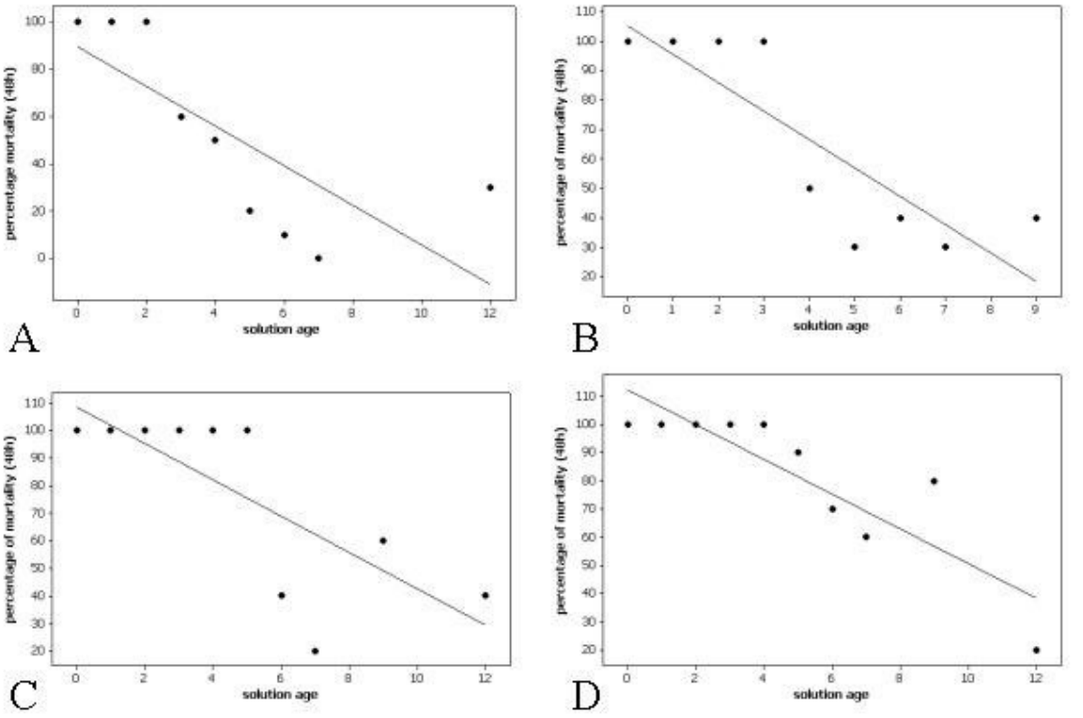


**Figure 1.** Scatter plots showing relation of used coffee ground suspension age and percentage larval mortality in 48 h treatment. Suspension concentrations: A. 75mg/ml, B. 150mg/ml, C. 225mg/ml and D. 300mg/ml.

In both experiments, we observed a variation in the treatment time that was necessary to produce 100% mortality as the suspension age increased, but in the Second Experiment, in 225 and 300 mg/ml concentrations, as the suspension age increased, the treatment time required for 100% mortality decreased.

Previous data on treatments of *A. aegypti* eggs with UCG in the concentration 50 or 100 mg/ml showed that this substance blocks the development in the larval stage. Even when larvae do not die in three or four days, the development is arrested in this stage (Laranja *et al.* 2003). In the same study UCG also affected developmental time increasing it.

In the present study, the effect of UCG on LM was analyzed in Experiments with two methods of treating the larvae: (a) the larvae were set free in the medium so that they could enter into contact both with UCG grains deposited at the bottom of the recipient and with the UCG suspension (the supernatant liquid) or (b) they were maintained in a fine-mesh sieve preventing them from entering into contact with the deposit, but immersed in the liquid. The objective of these different

**Table 1 -** Effect on *Aedes aegypti* larval mortality of used coffee ground (UCG) suspensions with different concentrations and ages after preparation, in two experiments, the First with larvae free in the medium and the Second, with larvae inside a sieve immerse in the medium supernatant. Food was not added in the tests of the experiment 1 (-), but was added to some tests of experiment 2 (+).

| Exp. | Food | UCG (mg/ml) | N | total number of tests | Larval Mortality | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 100% | | | < 100% | | | | |
| | | | | | number of tests (%) | suspension age (days) | range treat. time (days) | number of tests (%) | suspension age (days) | mortality range (%) | range treat. time (days) | number of adults produced |
| 1 | - | 75 | 130 | 9 | 3 (33) | 0 – 2 | 1 – 2 | 6 (67) | 3 – 7; 12 | 70 – 90 | 5 – 13 | 12 |
| | | 150 | 130 | 9 | 4 (44) | 0 – 3 | 1 – 2 | 5 (56) | 4 – 7; 9 | 60 – 80 | 9 – 14 | 13 |
| | | 225 | 140 | 10 | 6 (60) | 0 – 5 | 1 | 4 (40) | 6; 7; 9; 12 | 60 – 90 | 7 – 11 | 10 |
| | | 300 | 140 | 10 | 9 (90) | 0 – 7; 9 | 1 – 9 | 1 (10) | 12 | 60 | 8 | 4 |
| Control | | water | 50 | 1 | 0 | - | - | 1 | 0 | 70 | 30 | 15 |
| 2 | - | 75 | 60 | 6 | 3 (50) | 0; 3; 15 | 4 – 19 | 3 (50) | 2; 7; 9 | 70 – 80 | 14 – 34 | 7 |
| | | 150 | 70 | 7 | 3 (43) | 0; 3; 15 | 2 – 14 | 4 (57) | 2; 6; 8; 9 | 60 – 90 | 9 – 13 | 8 |
| | | 225 | 80 | 8 | 7 (88) | 0; 2; 3; 6; 11; 12; 15 | 2 – 9 | 1 (12) | 13 | 80 | 16 | 2 |
| | | 300 | 100 | 10 | 9 (90) | 0; 2; 3; 6; 8; 9; 11; 13; 15 | 1 – 11 | 1 (10) | 12 | 80 | 8 | 2 |
| | + | 75 | 20 | 2 | 0 (0) | - | - | 2 (100) | 1; 4 | 70; 90 | 8 – 12 | 4 |
| | | 150 | 20 | 2 | 1 (50) | 1 | 4 | 1 (50) | 4 | 90 | 9 | 1 |
| | | 225 | 20 | 2 | 1 (50) | 1 | 10 | 1 (50) | 4 | 60 | 11 | 4 |
| | | 300 | 20 | 2 | 2 (100) | 1; 4 | 2 | 0 (0) | - | - | - | - |
| Control | - | water | 30 | 1 | 0 | - | - | 1 | 0 | 27 | 37 | 22 |
| | + | water | 20 | 1 | 0 | - | - | 1 | 0 | 0 | 9 | 20 |

Exp. = experiment; treat. = treatment; N = number of larvae in each test.

**Table 2 -** Mortality (%) of *Aedes aegypti* in used coffee ground (UCG) suspensions with different concentrations and ages after preparation, in the First Experiment (larvae free in the medium). Food was not added. MT = Maximum Time (days) of mosquito surviving in the breeding sites; P = Mosquito death in the pupa stage. Each test started with ten larvae, except the control (50 larvae).

| UCG (mg/ml) | Test | Suspension age (days) | Mortality percentage in the treatment time (days) | | | | | | | | | | MT (days) | Number of adults |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | |
| 75 | 1 | 0 | 98 | 20 | - | - | - | - | - | - | - | - | - | - |
| | 2 | 1 | 80 | 100 | - | - | - | - | - | - | - | - | - | - |
| | 3 | 2 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 4 | 3 | 50 | 60 | 60 | 60 | 70 | 70 | 70 | 70 | 70 | 70 | 10 | 3 |
| | 5 | 4 | 50 | 50 | 50 | 60 (1P) | 90 | - | - | - | - | - | - | 1 |
| | 6 | 5 | 20 | 20 | 20 | 20 | 50 | 60 | 60 | 60 | 70 | 80 | 13 | 2 |
| | 7 | 6 | 10 | 10 | 10 | 10 | 60 | 80 | 80 | 80 | 80 | 90 | 11 | 1 |
| | 8 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 80 | 80 | 11 | 2 |
| | 9 | 12 | 30 | 30 | 50 | 50 | 50 | 50 | 70 | 70 | - | - | - | 3 |
| 150 | 1 | 0 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 2 | 1 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 3 | 2 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 4 | 3 | 80 | 100 | - | - | - | - | - | - | - | - | - | - |
| | 5 | 4 | 50 | 50 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 70 | 14 | 3 |
| | 6 | 5 | 20 | 30 | 40 | 40 | 40 | 50 | 50 | 50 | 70 | 80 | 13 | 2 |
| | 7 | 6 | 40 | 40 | 40 | 40 | 50 | 50 | 80 | 80 | 80 | 80 | 13 | 2 |
| | 8 | 7 | 20 | 30 | 70 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 10 | 2 |
| | 9 | 9 | 20 | 40 | 50 | 50 | 50 | 50 | 60 | 60 | 60 | - | - | 4 |
| 225 | 1 | 0 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 2 | 1 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 3 | 2 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 4 | 3 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 5 | 4 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 6 | 5 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 7 | 6 | 30 | 40 | 70 | 70 | 70 | 70 | 80 | 80 | 80 | 80 | 11 | 2 |
| | 8 | 7 | 10 | 20 | 50 | 80 | 80 | 80 | 80 | 90 | - | - | - | 1 |
| | 9 | 9 | 20 | 60 | 60 | 60 | 60 | 60 | 70 | 70 | 70 | - | - | 3 |
| | 10 | 12 | 40 | 40 | 50 | 50 | 50 | 60 | 60 | - | - | - | - | 4 |
| 300 | 1 | 0 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 2 | 1 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 3 | 2 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 4 | 3 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 5 | 4 | 100 | - | - | - | - | - | - | - | - | - | - | - |
| | 6 | 5 | 90 | 90 | 100 | - | - | - | - | - | - | - | - | - |
| | 7 | 6 | 60 | 70 | 70 | 80 (1P) | 100 | - | - | - | - | - | - | - |
| | 8 | 7 | 40 | 60 | 60 | 70 | 70 | 80 (1P) | 90 | 90 | 100 | - | - | - |
| | 9 | 9 | 80 | 80 | 80 | 80 | 80 | 90 | 100 | - | - | - | - | - |
| | 10 | 12 | 20 | 20 | 20 | 20 | 20 | 30 | 50 (2P) | 60 (1P) | - | - | - | 4 |
| Control | 1 | 0 | 8 | 14 | 20 | 34 | 34 | 34 | 34 | 36 | 36 | 36 | 30 | 15 |

treatments was to test the supposed "mechanical effect" of UCG suggested by FUNASA (FUNDAÇÃO NACIONAL DE SAÚDE do Ministério da Saúde) in the Technical Report published on the internet, on 21/11/2001 (no more available), concerning the use of UCG, that had been propagated in the media.

The present results shows that the effect of UCG on producing 100% LM is independent of contact between larvae and the deposit at the bottom of the container. The mortality actually seems to be due to toxic compounds of UCG present in both the supernatant liquid and the deposit. UCG includes, in addition to caffeine, anti-physiological components such as tannins, chlorogenic acid, caffeic acid and potassium in excess (Brenes 1979). Laranja *et al.* (2003) considered that some of these components of UCG, in addition to caffeine, might be involved in the detrimental effects on *A. aegypti* because the effects of UCG on the esterase pattern differed from those of caffeine when used isolated. In fact, data in literature shows that tannins have larvicidal activity (Silva *et al.* 2004). The property of the tannins to interact with proteins makes them highly toxic (Simões *et al.* 2001, *in* Silva *et al.* 2004).

A difference between the First and the Second Experiments was observed as to the duration of the effect of UCG on LM. The efficiency of the lowest concentrations of UCG (75 and 150 mg/ml), evaluated in terms of the treatment time necessary to cause 100% LM was smaller in the Second Experiment (1 to 2 days, in the First Experiment; 4 to 19 in the Second Experiment). These observations suggest that, in low UCG concentrations, since the amount of toxic components is probably greater in the deposit than in the supernatant, direct contact with the deposit increases contact with the toxic agents. In addition, the larvae feed on the UCG grains (as shown by the observation of black material inside their digestive tract), and this certainly increases the effect. The movement up and down of the larvae in the medium also may help to distribute the toxic components in the experimental vial when they are free.

Also in the lower concentrations (75 and 150 mg/ml), in general, as the suspension age increased, the treatment time necessary for the larvae to die also increased (reaching 14 days in the First Experiment and 34 days in the Second Experiment). Apparently, UCG components, which are toxic for *A. aegypti* become degraded and lose their effect as time goes by.

In the two experiments, tests using the higher UCG concentrations (225 and 300 mg/ml) and no addition of food showed an increased percentage with 100% LM (60% and 90% in the First Experiment and 88% and 90% in the Second, respectively) and showed an increased duration of the UCG effect (reaching nine days in the First Experiment and 11 days in the Second Experiment for 300 mg/ml).

The statistical analysis of data obtained in the First experiment, considering the percentage of larval mortality in the first 48h treatment, in every UCG concentration, and the suspension age showed inverse linear correlation coefficients, reinforcing the general observation that as UCG suspension age increased, the efficiency decreased, but more slowly in the greater concentrations.

The UCG medium at 300 mg/ml concentration was the most efficient for treatment of larvae. In both experiments, adults began to be produced in this medium only when the suspension was 12 days old. Besides, the number of adults produced in both cases, after this time, in tests without addition of food was smaller than that produced in the media with the lower UCG concentrations (75 and 150 mg/ml) during the entire time of treatment.

Normally, in natural breeding sites, larvae feed on aquatic microorganisms (bacteria, algae and yeasts), pollen, molts, carcasses of dead larvae and a wide variety of organic detritus that exists in the medium (Jones 1978). In the laboratory, because tap water is used, bacteria and carcasses of dead larvae are the main food source when fish food is not added. In the present study, the addition of food to UCG media did not alter the results substantially. In UCG at 300 mg/ml (aged one and four days), no adult was produced when fish food was added. In the lower concentrations, there was, similarly, no increase in the number of adults produced, when compared with the tests without the addition of food.

It is interesting to note that, in the Second Experiment (larvae in the sieve), adults were not produced in the three smaller concentrations (75, 150 and 225 mg/ml) of 15-day-old suspensions and in the 300 mg/ml concentration aged 13 days. This result may be attributed to water evaporation in the experimental breeding sites, which were only covered with a fine-mesh fabric. The evaporation reduced the amount of water, which may have impaired the survival of the larvae inside the sieve.

In the controls without addition of food, the larva – adult developmental time increased (30 and 37 days), in comparison to the control with addition of food (9 days). The low productivity of adults in the control of the First Experiment may be due to the fact that L3 larvae (more sensitive to the small availability of food) were predominantly used in that test.

The present results reinforce those obtained by Laranja *et al.* (2003) showing that UCG causes larval mortality in *A. aegypti* and then may be considered a potential agent for alternative control, when used in the appropriate concentration and with the addition of new UCG suspension at appropriate intervals. The present study shows that 300 mg/ml is a secure concentration. In relation to the interval, although the present results have shown that, at this concentration, 100% mortality occurs in media up to 9 days old, we consider it safer

to recommend the use of seven-day intervals for the addition of new UCG suspension.

Thus, although the elimination of the breeding sites remains being the best way to control *A. aegypti* population size, the present data reinforces the validity of considering UCG preparations as possible auxiliary in the alternative control of this mosquito. UCG might be recommended mainly to be used in gardens inside Bromeliaceae, in the dishes under vases (when the dishes cannot be discarded) and over the land in the vases. UCG has the advantage of being free of cost (it is normally through out after the drink preparation) and used by many people as fertilizer for plants.

**Acknowledgements**

Thanks are due to SUCEN (Superintendência de Controle de Endemias) of São José do Rio Preto - SP, for providing mosquitoes, to Dr. Antônio José Manzatto for help with the statistical analysis and to the Zoology and Botany Department of IBILCE for providing the mice for feeding the mosquitoes.

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