

**EFFECTS OF DIFFERENT COFFEE EXTRACTS ON
THE EGG FERTILITY AND LIFESPAN OF DENGUE
VECTORS (*Aedes albopictus* AND *Aedes
aegypti*) (DIPTERA: CULICIDAE)**

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ABSTRACT

This study was conducted to examine the impacts of various extracts of coffee on the egg production, aedes fertility and adult lifespan in two dengue vectors. Coffee is known for its chemical richness that is so far no resistance in insects was documented. For this purpose, two types of extracts (crude and used) from two types of coffee (fresh and roasted) of *Coffea canephora* (Robusta coffee) were used. We compared the effects of the extracts on the egg hatching responses and the longevity of dengue vectors; *Aedes albopictus* and *Aedes aegypti*. Overall, the roasted coffee extracts tend to have more negative impact than the fresh coffee extracts in reducing the hatching success. Crude extracts of both fresh/green and roasted coffee seem to

exerts more chemicals and appears to be more acidic than the used extracts in which resulted in reduced rate of larval eclosion. Longevity statistically has no difference among coffee extracts. However, based on the overall observation on the longevity of the adult and the difference in the longevity suggested that the exposure of the coffee to the dengue vectors during the embryogenesis does reduce the longevity of the survived adult by few days difference.

Keywords: Coffee, egg hatching response, mosquito control, *Aedes albopictus*, *Aedes aegypti*

ABSTRAK

Kajian ini dijalankan untuk mengkaji kesan pelbagai ekstrak terhadap penghasilan telur dan jangka hayat terhadap dua vektor denggi. Sehingga kini, tiada kajian tentangan yang direkodkan oleh serangga terhadap kopi. Oleh sebab itu, dua jenis ekstrak kopi (mentah dan terpakai) daripada dua jenis kopi *Coffea canephora* (segar dan panggang) digunakan untuk membandingkan kesan ekstrak terhadap tindakbalas penetasan telur dan jangka hayat vector denggi; *Aedes albopictus* dan *Aedes aegypti*. Keseluruhannya, ekstrak kopi panggang mencatat kesan negatif terhadap pengurangan penetasan berbanding kopi segar. Ekstrak mentah oleh kopi segar/ hijau atau panggang menghasilkan lebih banyak kimia dan bersifat asid dan mengurangkan kadar penetasan larva berbanding ekstrak terpakai. Kadar jangka hayat tidak menunjukkan sebarang perbezaan antara ekstrak kopi. Namun begitu, pemerhatian secara menyeluruh, menunjukkan perbezaan terhadap kadar jangkahayat dimana, pendedahan vektor denggi terhadap kopi sewaktu fasa embryogenesis mengurangkan jangka hayat dewasa untuk beberapa hari.

Kata kunci: Kopi, tindakbalas penghasilan telur, pengawalan nyamuk, *Aedes albopictus*, *Aedes aegypti*

INTRODUCTION

It has been suggested that there are at least 1000 chemicals found in one cup of coffee (Farah, 2012; Maranhon *et al.*, 2003). In addition, 950 more new compounds that is formed after roasting according to Farah (2012). Due to this chemical richness, research has been conducted by scientists to know any useful biological compounds and any possibilities of recycling it especially when the previous studies has proven that caffeine has insecticidal properties which can helps to reduce the population of insect pests and vectors. Furthermore, mosquito has been a major nuisance to humankind and is able to transmit fatal diseases like dengue and malaria. Most recently, a huge break occurs in Malaysia this early 2014 where the number of dengue cases increases more than 4 folds in 2013 (WPRO 2014). So, it has become a major concern on how to eliminate these vectors especially when the use of insecticide has increased the insecticide resistance and no specific dengue vaccine available currently (Schmitz *et al.*, 2011). However, no study has been conducted on effect of coffee chemistry towards embryogenesis in dengue vectors. This is important to investigate because the egg stage can spread the virus due to the ability to transmit it through vertical transmission. The finding of other safer alternative control agents is very important and thus became the main purpose in many research and studies. In this experiment, coffee extract is tested to know its effects on the egg fertility and the lifespan of the mosquito-borne virus; *Aedes aegypti* and *Aedes albopictus*.

MATERIALS AND METHODS

Experiment 1 (Egg hatching experiment)

The experimental egg was obtained from the gravid female of colony maintenance. The experiment was done by transferring gravid female into custom made oviposition device containing

experimental substrate for egg ovipositioning. Before transferring the gravid female, the substrate was first soaked in the extract/water for 30 minutes to ensure the substrate can take up the treatments (extracts/water). 10 replicates were prepared for each treatment RCC (crude extract from roasted coffee), RCU (used extract from roasted coffee), FCC (crude extract from fresh/green coffee), FCU (used extract from fresh/green coffee) and CTL (water-soaked substrates (control)). Then, all the females were left for 3 days in the oviposition device to lay the eggs on the given substrate. Following the 3 days, the females were released back into the cage and the substrate was taken out together with the eggs. The eggs were counted under the microscope and the value was recorded. It was then left for 3 days to dry under room temperature and soaked in water for 24 hours. The number of egg hatched was recorded for the calculation of rate of egg hatching (%).

$$\text{Rate of hatching} = \frac{\text{no of egg hatched}}{\text{initial no of eggs}} \times 100$$

Experiment 2 (Longevity experiment)

All the larvae derived from experiment 1 (RCC, RCU, FCC and CTL) were allowed to developed into adult. Once they had reached the pupal stage, the pupae were transferred into a temporary mosquito cage until they immersed as adults and were transferred into the test cagelater on (10 male and 10 female for each treatment RCC, RCU, FCC and CTL). Sucrose solution was prepared in a large amount so that the adult gets continuous supply. However the adult that has successfully emerged was not given blood feeding to avoid bias because blood fed females might get more energy and protein to survive longer. All the adult mosquitoes, males and unfed blood females was checked every day to see how many dies and the value was counted and recorded until all the adult died. The longevity can be calculated by summing up the days when the egg hatched until the adult dies.

RESULTS AND DISCUSSION

The overall observation from the results of this study suggested that the presence of coffee extract has significant effects and is not suitable for the viability of the eggs from both *Ae. albopictus* and *Aedes aegypti* which resulted in reduced hatching rate success (Figure 1). However this negative effect of coffee on egg viability was not observed when the extract originated from used coffee extractions (Figure 1).

It was found that roasted coffee extracts tend to have more negative impact than the fresh coffee extracts in reducing the hatching success (Figure 1). The fact that the RCC was more negative than FCC is more likely due to the fact that the chemicals that is presence in coffee after roasting process is more than in fresh/green coffee where approximately 950 new compounds is found after roasting (Farah, 2012). The high roasting temperature had resulted in some chemical compounds to degrade and some other new compounds to formed (Farah, 2012). And it is possible that there are a number of chemicals out of that few hundred that are capable of inducing mortality of the pharate larvae inside the egg especially when the pharate larvae need to undergoes moisture uptake process during the hardening of the chorion (Dieng *et al.*, 2006; Strickman, 1980). These pharate larvae might as well take up the toxic substances and accumulate it. The eggs that developed in RCC, hatched at a considerably lower rate than in FCC due to more uptakes of toxic substances and exposure to leachetes from coffee substrates during the embryogenesis (Figure 1).

The other factor is also due to the released labile substances from the RCC which act as hatching inhibitors by depleting the bacteria population that responsible for the favourable gradient of depletion of dissolved oxygen which consequently resulting in egg hatching failure (Dieng *et al.*, 2006). Indeed, it could be suggested that the release of the labile substances is likely to be higher in RCC than in FCC where the

significant negative impact on the hatching rate is higher in RCC.

From chemical analysis, we found that the turbidity and acidity between treatments was different. The crude extracts are more acidic than the used extracts. The increased in acidity is associated with the first extractions of coffee extracts where the chemicals are more concentrated than the second extraction (used extracts). Such variation in acidity might be influencing the outcome of a decreased response pattern in hatching rates of both vectors. This acidic environment disrupts the ionic regulation of the eggs which consequently reduced the larval eclosion (Rowe *et al.*, 1988; Tabak *et al.* 1991).

On the other hand, the used extracts of coffee; be it RCU or FCU on both *Aedes* species shows no significant difference as to hatching rate in CTL (Figure 1). It was probably because the used form of coffee has lost most of its chemicals to the crude extract during the first extraction (Tango, 1971).

As for the longevity experiment, even though statistically, there was no significant difference shown between the treatments and control, the differences are considerable because from the results obtained, there is a decreasing trend of number of days of survival of the adult derived from the treatments; they tend to live shorter than their peers that came from eggs that were maintained in a water environment (CTL). This means that the coffee does affects the longevity of the mosquitoes in a means that it shortened up the lifespan of the adult even just by a few days of difference (Figure 2).

As for the comparison of longevity between the two species of dengue vectors; *Ae. aegypti* and *Ae. albopictus*, the result shows that there was no significant difference between the two and the reduction pattern of longevity is relatively the same. However, it is important to remark that the longevity of both the dengue vectors was affected by the coffee treatment (Figure 2).

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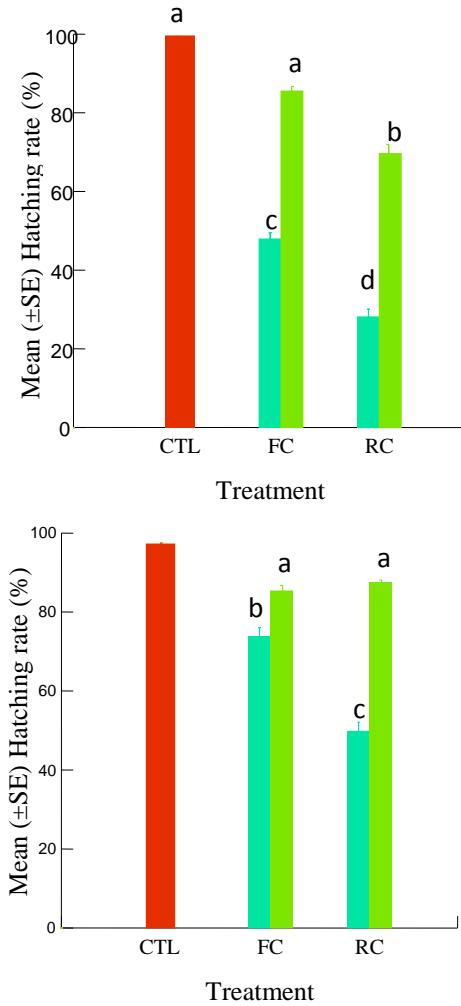


Figure 1. Hatching responses of *Ae. albopictus* (left) and *Ae. aegypti* (right) following egg exposure to different forms of extractions from fresh (FC), roasted (RC) robusta coffee and control (water only)

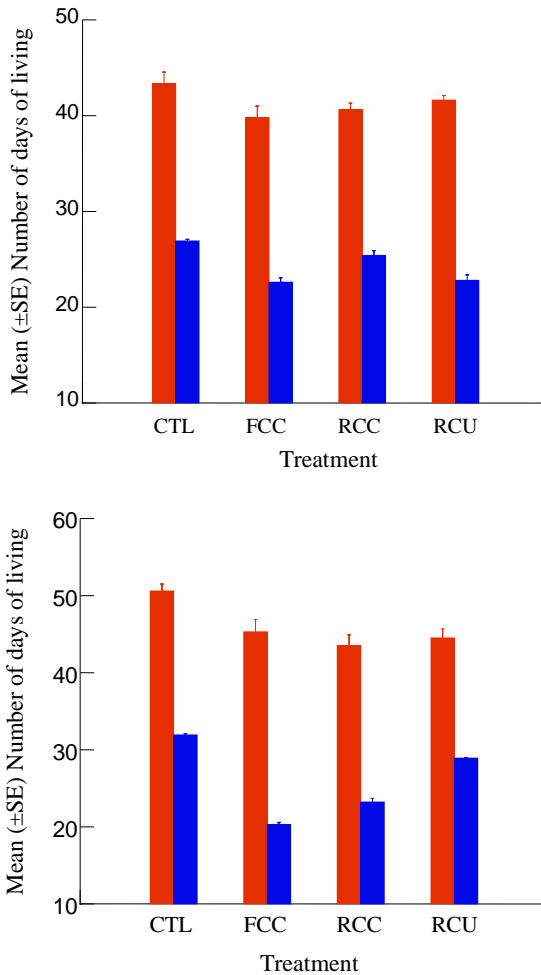


Figure 2. Longevity of *Ae. albopictus* (left) & *Ae. aegypti* (right) following exposure to different forms of extractions from crude extract of fresh coffee (FCC), crude extract of roasted coffee (RCC), used extract of roasted coffee (RCU) and control (water only) during embryogenesis.

CONCLUSION

Even though the elimination of the breeding grounds remains the best way in controlling dengue vector population, the results from this data has strengthen the plausibility of coffee as an alternative control to this mosquito. Our results are at least a proof of concept that coffee represents a potential new way to interfere with the dengue vector's life cycle mainly by decreasing the hatching rate of the eggs and reducing the longevity of the adult.

However, it seems that the used coffee extract does not really compelling the effects on the egg fertility of the dengue vectors. Thus, this observation needs further study especially when previously in studies done by Laranja *et al.*, (2003) and Derraik *et al.*, (2005), they found that in nutrient-poor water conditions, used coffee extracts could favour the larval survivorship because the presence of the used coffee extracts which is not as strong as crude extracts of coffee will promote the bacterial and algal growth as well as the content itself which has fatty acids, amino acids and few more other nutrients that could serve as food in replacing the poor-nutrient conditions in the breeding area. The application of pure crude extract of coffee might not be practical as part of a wide-scale project because coffee as we know is a daily consumed beverage and only the waste is thrown away like the used coffee ground. So, one way suggested is that maybe the active components in the coffee can be isolated such as caffeine and tannin which have larvicidal activity (Silva et al., 2004), and also pyridinium formate that possess antiviral activity (Tsujiimoto *et al.*, 2010).

ACKNOWLEDGEMENTS

Million thanks to my supervisor Assoc Prof Dr Hamady Dieng and Dr Abdul Hafiz Ab. Majid for his comments and valuable suggestions on this project and USM School of Biological Sciences for technical and administrative assistance. The authors would also like to thank KPM- FRGS (203/PBIOLOGI/6711360) for a financial support.

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