

Effects of vitamin B fortified sucrose solution on the longevity and reproductive potentials of laboratory-bred *Culex quinquefasciatus* Say adult

Tan, S.B.¹, Nazni, W.A.¹, Misni, S.², Zuraini, Z.² and Lee, H.L.^{1*}

¹Medical Entomology Unit/WHO Collaborating Centre for Vectors, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia

²School of Distance Education, Universiti Sains Malaysia, Penang, Malaysia

*Corresponding author e-mail: leehl@imr.gov.my

Received 3 May 2015; received in revised form 8 September 2015; accepted 15 September 2015

Abstract. Laboratory colonised mosquitoes are usually maintained on vitamin B complex fortified sucrose solution, however only few studies were conducted to evaluate the effects of such practice. This study aimed to determine the effects of different concentrations of sucrose solution fortified with and without 1% vitamin B complex on the longevity and reproductive potential of adult female of a local strain of *Culex quinquefasciatus* Say. Two arms of studies were carried out separately and each arm was triplicated. In one arm, concentrations of sucrose solution at 0%, 1%, 3%, 5% and 10% fortified with 1% Vitamin B complex were fed to the mosquitoes, while in the other arm, similar sucrose concentrations were used but without 1% vitamin B complex. Adult *Cx. quinquefasciatus* maintained on 5% sucrose solution fortified with 1% vitamin B complex exhibited significant extended vitality and longevity in stimulating ovarian development, compared with other vitamin fortified sucrose concentrations ($p < 0.05$). The vitality and longevity of F₀ and F₁ males were 76.67±2.19 days and 57.67±8.19 days respectively. The F₀ females survived the longest duration of 107.67±5.61 days and the F₁ females survived 90.67±12.47 days with higher number of eggs laid, i.e. 1427.67±62.89 eggs at a higher hatchability rate of 57.05±8.39% or 814.49 eggs hatched. Thus, 5% sucrose solution fortified with 1% Vitamin B complex should be used to produce colonies of homogenous mosquitoes as this exerts positive biological effects on laboratory-bred *Cx. quinquefasciatus*.

INTRODUCTION

Dietary sugar is a major food source for adult mosquitoes as flight fuel, immediate metabolic needs, survival, longevity, and host finding (Michael, 2006). Laboratory-bred mosquitoes have been fed on a wide range of concentrations of sucrose solution from 1% to 50% (Nayar & Sauerman, 1975). Laboratory colonised mosquitoes in our insectarium are usually maintained on vitamin B complex fortified sucrose solution, but only few studies were conducted to evaluate the effects of such a practice. All species of laboratory bred adult mosquitoes are reportedly fed with different concentration of sucrose solution from 1% to 50%. However, 10% sucrose

solution appears to provide the best results (Gerberg, 1970). To date, there is still no standard guideline or documentation to state the optimum sucrose concentration for adult viability and longevity. The insectarium at the Institute for Medical Research, Kuala Lumpur, is using 10% sucrose solution fortified with 1% of vitamin B complex as the adult energy food source. Other insectaria around the world such as the Walter Reed Army Institute of Research, U.S.A. is using 10% sucrose soaked cotton balls for the newly emerged adults, while others are using 1%, 2%, 4% or 8% glucose solution (Schwartz & Koella, 2002), 5% sucrose solution (Hemingway, 2008), 6% glucose solution (Smallegange *et al.*, 2005; Manda *et al.*, 2007

and Sumba, 2007) and 10% glucose solution (Calheiros *et al.*, 1998 and Geier *et al.*, 1999).

This study aimed to determine the effects of different concentrations of sucrose solution fortified with and without 1% vitamin B complex on the longevity and reproductive potential of adult females of a local strain of *Cx. quinquefasciatus* Say.

MATERIALS & METHODS

Study site

The study was conducted from 2006 to 2009 in the main laboratory of the Medical Entomology Unit/WHO Collaborating Centre for Vector, Infectious Disease Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia.

Mosquitoes tested

Laboratory-bred *Cx. quinquefasciatus* were used in this study. The mosquitoes were maintained with 10% sucrose solution fortified with 1% Vitamin B complex in the insectarium of IMR at temperature and relative humidity of $27\pm 2^{\circ}\text{C}$ and $80\pm 2\%$ respectively with a photoperiod of 12:12 hours (L:D). Male and female pupae from the colony were selected and kept in individual tube accordingly. A total of ten cages each measuring 30cm x 30cm x 30cm were used and properly labeled. On emergence into adults, ten females and thirty males (ratio 1:3) were put together in each cage labeled as F0. F0 Females were given only a single blood meal throughout this study. The blood of laboratory white mice, *Mus musculus* was used as a protein source for egg development.

Concentrations of sucrose solution

Concentrations of sucrose solution at 0%, 1%, 3%, 5% and 10% fortified with and without 1% Vitamin B complex were placed in the appropriate cages accordingly. Males and females were then given unlimited access of the above different concentrations of sucrose solution.

Tests

Two arms of studies were carried out separately and each arm was triplicated. In

one arm, concentrations of sucrose solution at 0%, 1%, 3%, 5% and 10% fortified with 1% Vitamin B complex were fed to the mosquitoes, while in the other arm, similar sucrose concentrations were used but without 1% vitamin B complex. For each replicate, ten cages were used; five cages for F0 and another five cages for F1. A total of 1,200 mosquitoes (300 females and 900 males) were used in this study. Daily observation of F0 gonotrophic cycle was conducted after a single blood meal. F0 dead adult mosquitoes were removed and counted daily from each cage. F1 larvae, pupae and adult % mortality, mean hatching rate, % mortality of hatched larvae, number of unhatched eggs, F0 and F1 male and female % emergence and % vitality & longevity were also recorded. F1 adults were given weekly blood meal until all females were dead. Measurement and counting of egg raft and the number of eggs per raft were done under a stereo microscope. The number of hatched & unhatched eggs and live & dead larvae were counted and recorded.

Ethical considerations

Approval to use mice for entomological research was obtained from the Ministry of Health [Ref No KKM.KPK.5305.20/11 Jld 13(37), 2006 Nov 24], in accordance with the Destruction of Disease Bearing Insects Act, 1975.

Statistical analysis

All data were expressed as the mean \pm SD. A value of $p < 0.05$ was considered significant. Data obtained from the test were subjected to statistical analysis using a statistical software programme (SPSS® version 16.0; IBM, Armonk, NY).

RESULTS

Cx. quinquefasciatus fed on sucrose solution fortified with 1% Vitamin B complex (Arm 1) produced larger raft size ranging from $3.15\pm 0.63\text{mm}$ to $3.97\pm 0.46\text{mm}$ (Fig. 1) with 11.67 ± 2.91 to 18.33 ± 5.81 egg rafts (Fig. 2) and accounted for higher number of viable larvae ranging from 347.33 ± 79.70

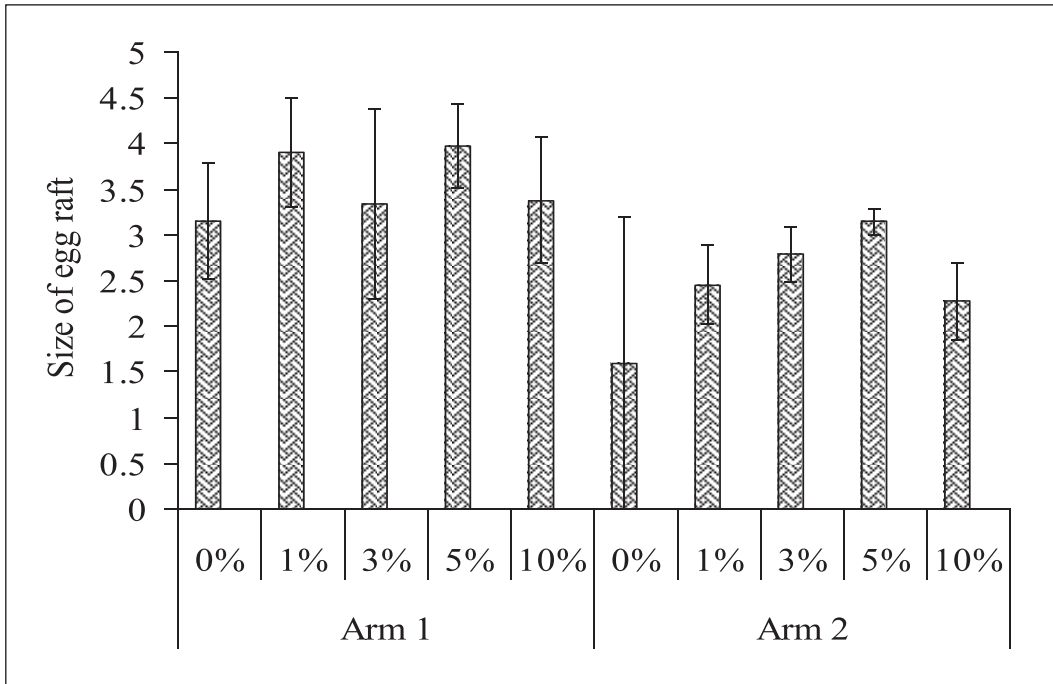


Figure 1. Comparison between two arms of studies on size of egg rafts of lab-bred *Culex quinquefasciatus*.

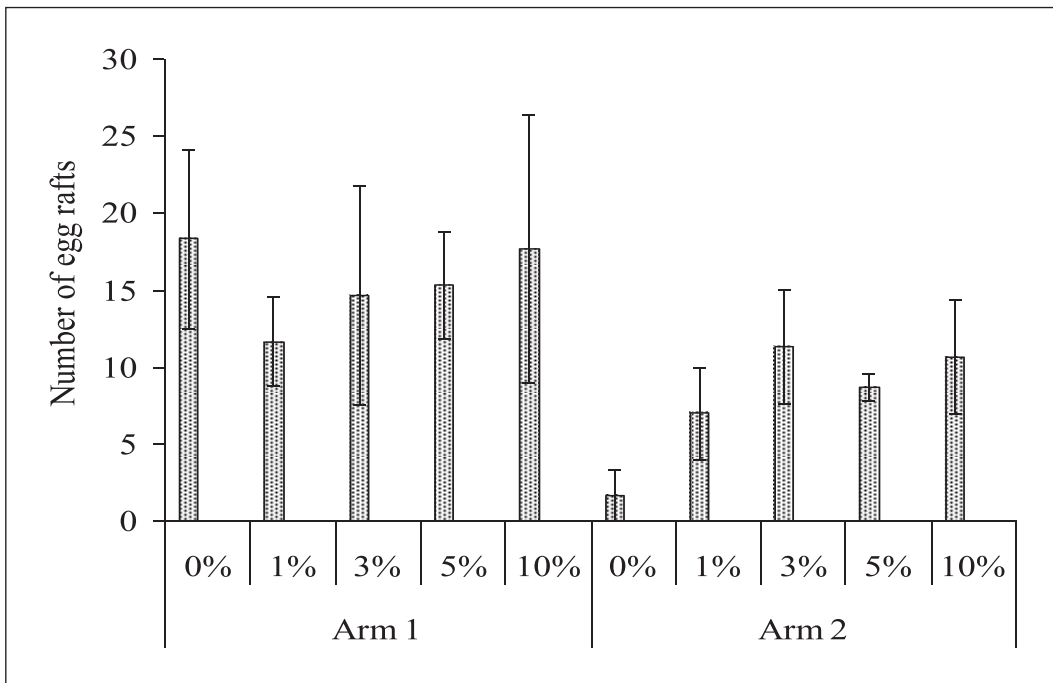


Figure 2. Comparison between two arms of studies on the number of egg rafts of lab-bred *Culex quinquefasciatus*.

DISCUSSION

(25.88±25.99%) to 941.33±228.80 (62.60±80.42%) (Fig. 3). On the other hand, it also accounted for the highest number of unhatched eggs ranging from 562.33±55.72 (37.40±19.58%) to 995.00±226.90 (74.12±74.01%) (Fig. 3).

Based on data obtained, 5% sucrose solution fortified with 1% Vitamin B complex (Arm 1) and 3% sucrose solution without 1% Vitamin B complex (Arm 2) exhibited significant differences ($p < 0.05$) in providing the optimum results. Adult females of *Cx. quinquefasciatus* fed with a single blood meal followed by unlimited access of 5% and 10% sucrose solution fortified with 1% Vitamin B complex (Arm 1) (Fig. 4) were able to produce as many as 1427.67±62.89 eggs and 1601.00±253.79 eggs respectively.

A strong positive correlation ($r = 0.708$, $p < 0.01$) was observed between sucrose concentrations to the total number of eggs laid. No correlation was observed for adult vitality and longevity. Hence, as the sucrose concentration increased, the number of eggs laid also increased correspondingly.

Three percent sucrose solution fortified with 1% Vitamin B complex was significant ($p < 0.05$) in providing optimum results in adult male and female vitality and longevity (Fig. 1) but it was not significant ($p > 0.05$) for egg production (Fig. 4). Hence, it was not chosen to be used in the insectarium. Ten percent sucrose solution fortified with 1% Vitamin B complex was also not used in the insectarium due to its viscosity of the sucrose which coagulated easily after a few days emitting sourly smell and the need for frequent replacement of sucrose solution.

We considered many other aspects in choosing the optimum concentrations of sucrose solution to be used in the insectarium. Therefore, 5% sucrose solution fortified with 1% Vitamin B complex was chosen to be used in the insectarium. At 5% sucrose solution fortified with 1% Vitamin B complex, the F0 and F1 males survived for 76.77±2.19 days and 57.67±8.19 respectively (Fig. 5). The F0 females survived the longest duration

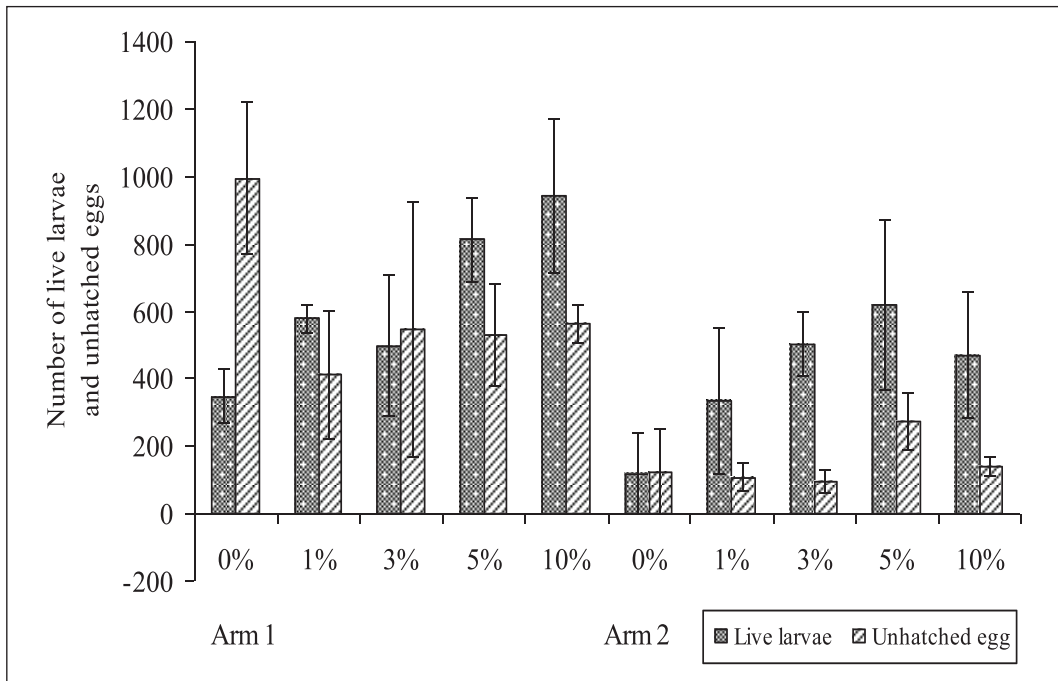


Figure 3. Comparison between two arms of studies on the number of live larvae and unhatched eggs of lab-bred *Culex quinquefasciatus*.

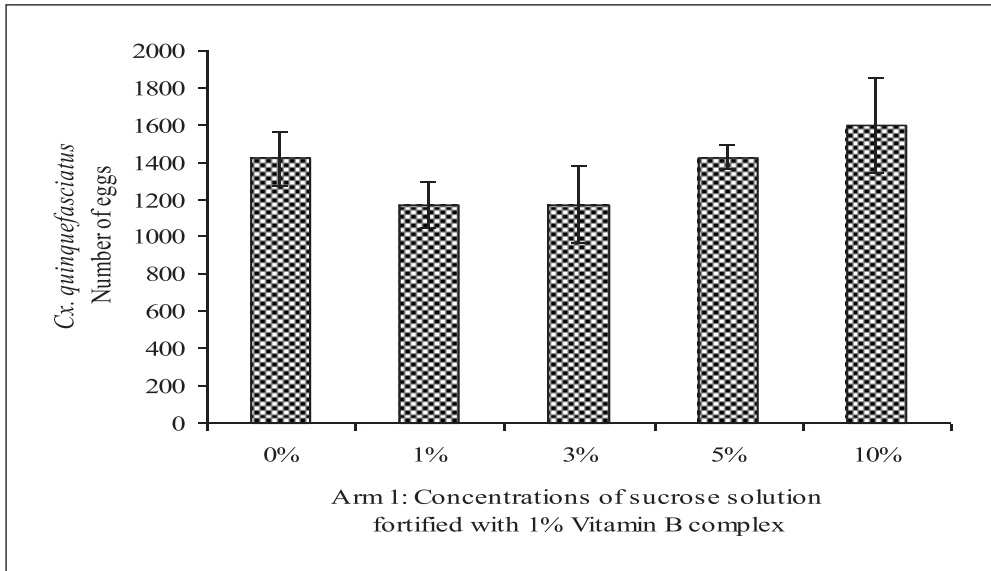


Figure 4. Effects of different concentrations of sucrose solution fortified with 1% Vitamin B complex on total number of eggs laid by adults of lab-bred *Culex quinquefasciatus*.

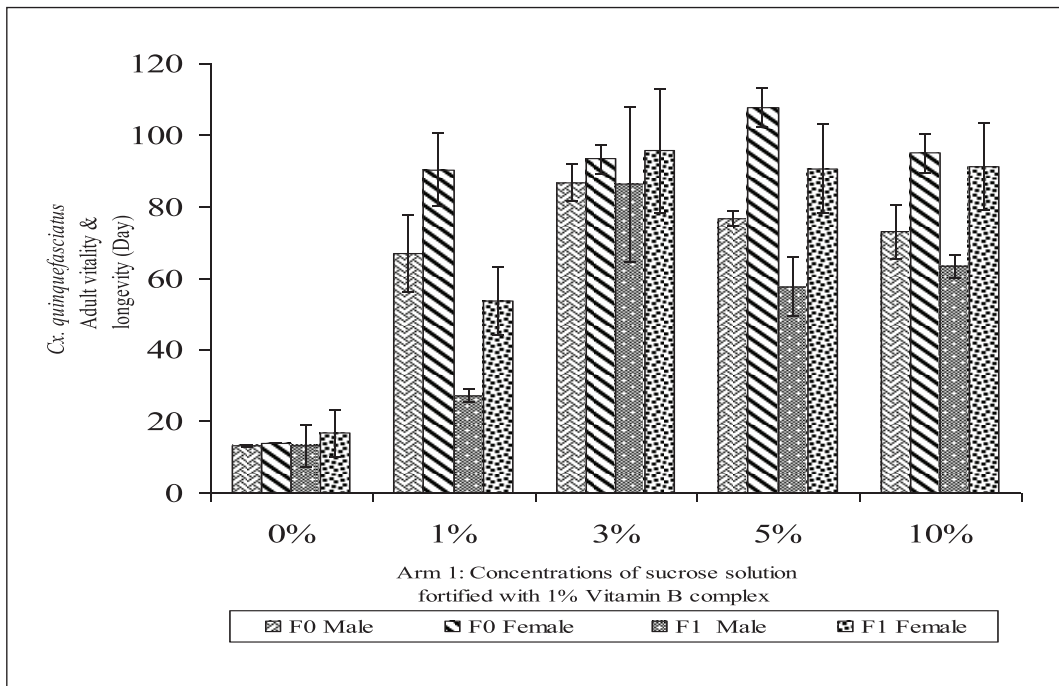


Figure 5. Effects of different concentrations of sucrose solution fortified with 1% Vitamin B complex on the adult vitality and longevity of lab-bred *Culex quinquefasciatus*.

of 107.67 ± 5.61 days and the F1 females survived 90.67 ± 12.47 days (Fig. 5) with higher number of eggs laid, i.e. 1427.67 ± 62.89 eggs (Fig. 4) at a higher hatchability rate

of $57.05 \pm 8.39\%$ or 813.33 ± 126.44 eggs hatched (Fig. 3 & 6). There were significant differences ($p < 0.05$) between the two arms of studies on few parameters on the

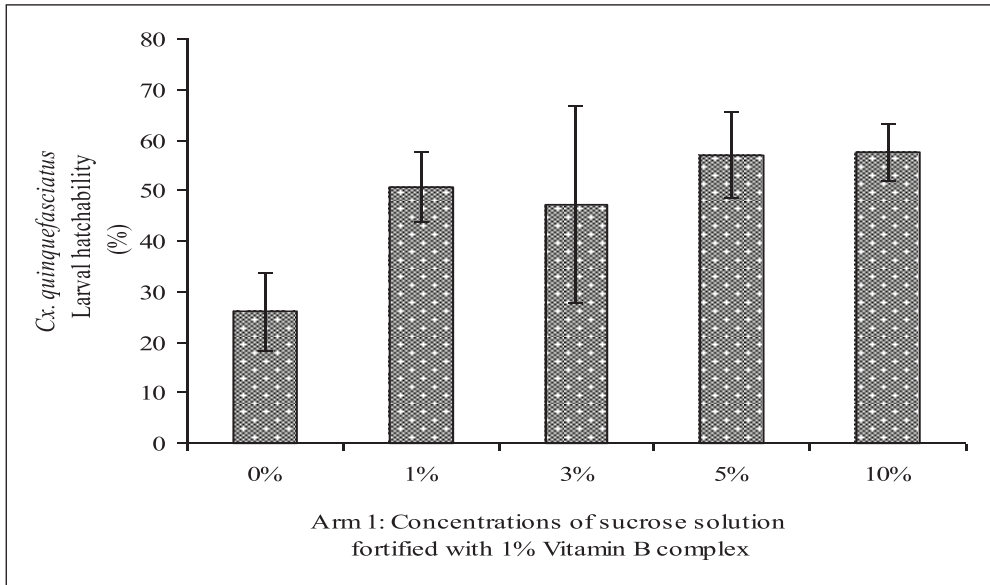


Figure 6. Effects of different concentrations of sucrose solution fortified with 1% Vitamin B complex on larval hatchability rate of lab-bred *Culex quinquefasciatus*.

biology and life history of lab-bred *Cx. quinquefasciatus*. Hence, we concluded that concentrations of sucrose solution fortified with 1% Vitamin B complex (Arm 1) exhibited optimum results by producing larger raft size from 3.15 ± 0.63 mm to 3.967 ± 0.46 mm (Fig. 1). It also accounted for higher raft count from 11.67 ± 2.91 rafts to 18.33 ± 5.81 rafts (Fig. 2) and a higher number of viable larvae from 347.33 ± 79.70 to 941.33 ± 228.80 larvae (Fig. 3).

Our findings on 3% sucrose solution without 1% Vitamin B complex exhibited that with only a single blood meal, the F0 females survived for 105.67 ± 8.97 days (Fig. 7) which concurred with the study done by Majid and Sinton (1933) that forty females of *Cx. fatigans* (= *Cx. quinquefasciatus*) survived for 105 days and that 5 other females of *Cx. fatigans* survived for up to 189 days with only a single blood meal and followed by unlimited access of sucrose solution (Table 1). The female mosquitoes were maintained under controlled temperature of 12-26°C with a relative humidity of 60-80%, which was quite similar to our study.

Laboratory survivorship data for sugar fed females for several of Florida's common mosquito species indicated species specific variation (Nayar & Sauerman 1975).

All species lived for about 24 hours post emergence without a sugar meal, but survival of mosquitoes fed once on 50% sugar solutions survived for 144 hours or 6 days (*An. quadrimaculatus*) to 336 hours or 14 days (*Ae. taeniorhynchus*). Our post emergence study showed that water fed (without sugar and Vitamin B complex) lab-bred male mosquitoes of F0 and F1 survived for 9.00 ± 4.40 days and 5.33 ± 5.55 days respectively, whereas F0 and F1 female survived for 10.00 ± 3.79 days and 5.33 ± 5.33 days respectively (Fig. 7). However, with the addition of 1% sucrose solution, the vitality and longevity of F0 and F1 male extended to 80.33 ± 2.60 days and 50.67 ± 14.75 days respectively and F0 and F1 females extended to 95.00 ± 7.64 days and 77.33 ± 13.29 days respectively (Fig. 7).

In general, smaller mosquito species were more efficient than larger species in utilizing their sugar reserves. Burkett (1998) found that survivorship length did not differ significantly for *Cx. nigripalpus* fed once on 10% solutions of glucose, sucrose, or melezitose. Assuming wild mosquitoes approach anything close to the 6-14 days of survival following a full sugar meal, it seems reasonable that wild mosquitoes can go at least 3-4 days without sugar feeding. Sugar

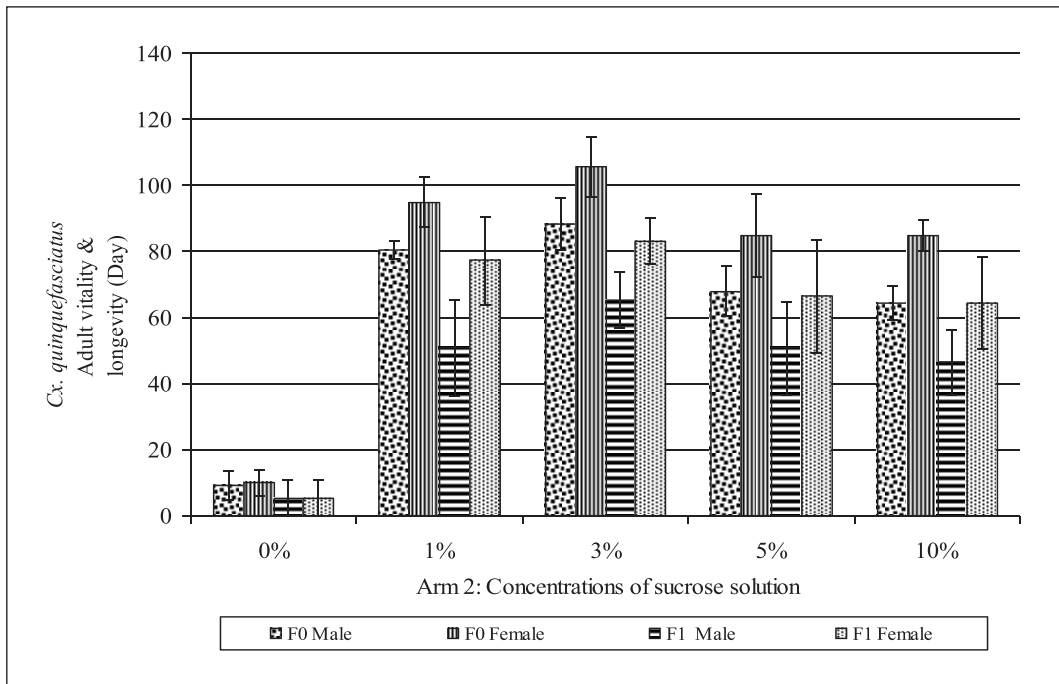


Figure 7. Effects of different concentrations of sucrose solution without 1% Vitamin B complex on the adult vitality and longevity of lab-bred *Culex quinquefasciatus*.

Table 1. Longevity of adult stage of *Culex pipiens* (Source: Horsfall, 1972)

| Sub-species | No. | Sex | Food | Temp (°C) | RH (%) | Longevity (Days) | Citation |
|-------------------------------------|-------|-----|----------------------|-----------|--------|------------------|------------------------|
| <i>Fatigans (=quinquefasciatus)</i> | 40 | ♀ | 1 blood meal & sugar | 12-26 | 60-80 | 105 | Majid & Sinton, 1933 |
| <i>Fatigans (=quinquefasciatus)</i> | 5 | ♀ | 1 blood meal & sugar | 12-26 | 60-80 | 189 | <i>Ibid.</i> |
| <i>Fatigans (=quinquefasciatus)</i> | – | ♀ | ? | 27 | 45 | 5 | Gill, 1921 |
| <i>Fatigans (=quinquefasciatus)</i> | 100 | ♀ | ? | 27 | 65-80 | 10+ | <i>Ibid.</i> |
| <i>Fatigans (=quinquefasciatus)</i> | ? | ♀ | Cage | ? | ? | 30 | Wanson & Nicolay, 1937 |
| <i>molestus</i> | ? | ♀ | No food | 22-25 | ? | 11 | Gaschen, 1932 |
| <i>molestus</i> | ? | ♀ | No food | 17-18 | ? | 11 | <i>Ibid.</i> |
| <i>molestus</i> | ? | ♀ | No food | 14-15 | ? | 12 | <i>Ibid.</i> |
| <i>pipiens</i> | ? | ♀ | Fruit | 8-10 | ? | 3-40 | Boissezon, 1930 |
| <i>pipiens</i> | ? | ♀ | Fruit | 8-10 | ? | 210+ | <i>Ibid.</i> |
| <i>molestus</i> | ? | ♀ | No food | 12-20 | ? | 6.2-13.3 | Tate & Vincent, 1936 |
| <i>molestus</i> | 60 | ♀ | Fruit | 12-20 | 70 | <i>Ibid.</i> | |
| <i>molestus</i> | 25 | ♀ | Fruit | 12-20 | 105 | <i>Ibid.</i> | |
| <i>molestus</i> | small | ♀ | Fruit | 12-20 | 175 | <i>Ibid.</i> | |

feeding frequency in wild populations has likely evolved to meet the needs of specific species as some species are known to feed at all stages of their gonotrophic cycle (Nasci & Edman 1984; Magnarelli 1978), but sugar feeding frequency data for wild population species remain unavailable. Sugar feeding is likely to vary by species, and depends on the mosquito's physiological and environmental conditions.

Since other researchers were using sucrose concentration of as high as 50%, future efforts on larger sample sizes on different species of mosquitoes and different concentrations of sucrose solution of 0% to 50% should be conducted to determine the efficacy of sucrose solution on the biology of all species of adult mosquito.

Acknowledgements. The authors thank the Director General of Health, Malaysia for permission to publish this paper and the Director, Institute for Medical Research, for support.

REFERENCES

- Burkett, D.A., Carlson, D.A. & Kline, D.L. (1998). Analysis of sugar meal composition of wild mosquitoes by gas chromatography. *Journal of American Mosquito Control Association* **14**: 373-9.
- Calheiros, C.M.L., Gilberto, F., Paul, W. & Eliana, M.M. (1998). Experimental infection of *Culex* (*Culex*) *quinquefasciatus* and *Aedes* (*Stegomyia*) *aegypti* with *Wuchereria bancrofti*. *Memorias do Instituto Oswaldo Cruz* **93**: 855-860.
- Geier, M., Bosch, O.J. & Boeckh, J. (1999). Ammonia as an attractive component of host odor for the yellow fever mosquito, *Aedes aegypti*. *Chemical senses*. Oxford University Press.
- Gerberg, E.G. 1970. (1970). Manual for mosquito rearing and experimental techniques. *American Mosquito Control Association Bulletin* No. 5 January 1970.
- Hemingway, J. (2008). Efficacy of etofenprox against insecticide susceptible and resistant mosquito strains containing characterized resistance mechanisms. *Medical and Veterinary Entomology* **9**: 423-426.
- Magnarelli, L.A. (1978). Nectar-feeding by female mosquitoes and its relation to follicular development and parity. *Journal of Medical Entomology* **14**: 527-530.
- Manda, H., Gouagna, L.C., Foster, W.A., Jackson, R.R., Beier, J.C., Githure, J.I. & Hassanali, A. (2007). Effect of discriminative plant-sugar feeding on the survival and fecundity of *Anopheles gambiae*. *Malaria Journal* **6**: 113.
- Michael, G.S. (2006). *Plant Systematics*. p182. Elsevier Academic Press.
- Nasci, R.S. & Edman J.D. (1984). *Culiseta melanura* (Diptera: Culicidae): population structure and nectar feeding in a freshwater swamp and surrounding areas in southern Massachusetts USA. *Journal of Medical Entomology* **21**: 567-572.
- Nayar, J.K. & Sauerman, D.M. (1975). The effects of nutrition on survival and fecundity in Florida mosquitoes. Part 1. Utilization of sugar for survival. *Journal of Medical Entomology* **12**: 92-8.
- Schwartz, A. & Koella, J.C. (2002). Melanization of *Plasmodium falciparum* and C-25 sephadex beads by field-caught *Anopheles gambiae* (Diptera: Culicidae) from Southern Tanzania. *Journal of Medical Entomology* **39**: 84-88.
- Smallegange, R.C., Qiu, Y.T., van Loon, J.J.A. & Takken, W. (2005). Synergism between ammonia, lactic acid and carboxylic acids as kairomones in the host-seeking behaviour of the malaria mosquito *Anopheles gambiae sensu stricto* (Diptera: Culicidae). *Chemical senses* **30**: 145-152.
- Sumba, L.A. (2007). Mediation of oviposition site selection in the African malaria. *Journal Cambridge Memorial Institute. Oswaldo Cruz* **97**: 359-362.