Development of an Alternative Low-Cost Larval Diet for Mass Rearing of *Aedes aegypti* Mosquitoes In Sri Lanka

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Abstract

Background

Aedes aegypti is a major vector of arboviruses that may be controlled on an area-wide basis using novel approaches such as Sterile Insect Technique (SIT) and Incompatible Insect Technique (IIT). Larval diet is a major factor in mass-rearing of *Aedes* mosquitoes for SIT and IIT programs. Therefore, current study aimed to evaluate the effects of two novel diets developed from dry fish powder on growth and development of immature stages and adult fitness-related characteristics of *Ae. aegypti* in Sri Lanka.

Method:

Three batches of 250 *Ae. aegypti* first instar larvae were exposed to three different larval diets as, standard dry fish powder (D_1), dry fish powder meal and brewer's yeast (D_2) and International Atomic Energy Agency (IAEA) reccomanded diet (D_3), separately. Morphometric and developmental parameters of 4th instar larvae, pupae and adult mosquitoes reared under different dietary treatments were measured. General Linear Model (GLM) was used for statistical analysis.

Results

Significant diet-based variations were observed in the head length, head width, thoracic length, thoracic width, abdominal length, abdominal width and total length ($F_{2, 87}$>4.811; $P < 0.05$) of *Ae. aegypti* larvae. The highest pupation success and the larval size were observed from the larvae fed with D_2 diet, while the lowest was reported from D_1. All adult morphometric parameters of adult male and female *Ae. aegypti* mosquitoes also denoted significant dietary variations, reporting the best sized adults from D_2 diet ($F_{2, 87}$>3.54; $P < 0.05$). Further, significantly higher fecundity and male longevity were also shown by the adult *Ae. aegypti* ($F_{2, 6}$>7.897; $P < 0.01$) reared under diet D_2.

Conclusion

Based on all the growth and developmental parameters, D_2 diet reported the best quality adult mosquitoes similar to the IAEA recomanded diet, while being more inexpensive. Therefore, larval diet D_2 could be recomaded as the ideal diet for for mass-rearing of *Ae. aegypti* for IIT and SIT-based vector control in Sri Lanka.

Background

Dengue is a mosquito-borne viral disease that has threatned morethan 129 countries in different parts of the world in recent years. About 390 million dengue viral infections occur every year and virtually 3.9 billion people live in dengue endemic countries [1]. Approximately, 1.8 billion people (more than 70%) at risk for dengue infection reside within the Western Pacific and South-East Asia Regions, approximately contributing to 75% of the global disease burden of dengue [2]. The number of dengue cases reported to WHO per yaer has increased, from 505,430 cases in 2010 to 3,312,040 in 2015. Meanwhile, annual deaths have increased upto 4,032 or higher in 2015 comapred to 960 in 2000 [1].

Sri Lanka has been affected by regular epidemics of Dengue Fever (DF) and Dengue Haemorrhagic Fever (DHF) for over two decades. Dengue Virus (DENV) infections have been endemic in Sri Lanka since the mid-1960s. DF was serologically confirmed in the island in 1962, while the first epidemic of dengue in Sri Lanka was experienced in 1980s [3]. Regular epidemics of dengue have been reported from Sri Lanka since 2009, with an average case burden of 35 000–45 000 per year. However, due to recent changes in virus serotype(s), Sri Lanka is facing a dramatic increase in the incidence of dengue leading to an alarming situation at present [4]. The highest number of dengue cases was reported in 2017 as 186, 101 with over 350 deaths [5].

In Sri Lanka, *Aedes aegypti* act as the primary vector of dengue, while *Aedes albopictus* contribute as the secondary vector. Due to the absence of an effective drug or vaccine for dengue, patient management and vector control are considered as the most

effective strategies to control dengue in the country [4]. Up to now different conventional methods are being used for dengue control, with emphasis on vector control. Environmental management, chemical based vector control methods, community participation and biological control could be identified as the current vector control strategies used for the suppression of *Aedes* vectors in Sri Lanka [6–7].

Significant adverse impacts on non-target populations, development of resistance to insecticides, higher financial costs and relative incompetence of chemical vector control approaches have encouraged the government entities to consider alternative methods for vector control in Sri Lanka [8]. Therefore, novel and innovative approaches such as the use of genetically modified mosquitoes, Sterile Insect Technique (SIT) and Incompatible Insect Technique (IIT) are being considered to effectively control dengue outbreaks in Sri Lanka[9].

As both IIT and SIT relies upon release of sterile male mosquitoes in large numbers, mass-rearing of *Aedes* vectors is one of the principal requirements in the process [10]. It is a challenging task as the production of mosquitoes in sufficient number and of adequate quality are cruicial for the success of IIT and SIT. Further, since the integration of the SIT into an areawide integrated pest management (AW-IPM) programme competes economically with other control techniques, the production of *Aedes* mosquitoes must be timely and cost effective [9].

In the mass rearing phase, mainly the rearing conditions and larval diet quality have a direct and often irreversible effect on adult traits [11]. According to Timmermann and Briegel [12], the larval diet should provide a wide range of nutrients to avoid the risk of deficiencies that could negatively affect both the rearing productivity and the fitness of the mosquitoes produced. When selecting a diet for mass rearing purposes, the components in the diet also play an important role. Since highly nutrious ingredients such as wheat germ, soy flour, ground beef, and chicken eggs are locally available at inexpensive prices, development of an optimum larval diet for mass rearing of *Aedes* mosquitoes with readily available ingredients would be more profitable [13].

In contrast, the International Atomic Energy Agency (IAEA) has recommended a specifically designed diet to be used for mass rearing, seeking to provide adequate nutritional components to ensure optimum rearing and adult insect quality. However, the ingredients of IAEA diet are expensive and limitedly available, despite the consistence in quality [14]. IAEA diet has been widely utilized for mass rearing of *Ae. aegypti* in many developing countries including Sri Lanka. However, IAEA diet should be either imported or locally prepared, both of which are costly [15]. Therefore, many countries are seeking to develop more economic alternative larval diets, which are readily available and provide the same nutritional properties to *Ae. aegypti* larvae [9]. Therefore, the current study was conducted to evaluate the performance of two locally developed larval diets with IAEA recommended diet to be used for mass rearing of *Ae. aegypti* in Sri Lanka, while ensuring optimum morphometric development and adult sexual competiveness, under laboratory settings.

Methods

Aquiring of Ae. aegypti **larvae**

Ae. aegypti eggs were obtained from the indoor insectary of the Molecular Medicine Unit (MMU), Faculty of Medicine, University of Kelaniya. Collected eggs were transferred to 1 L plastic trays with deoxygenated water in the Arthropod Containment Facility at the MMU and kept for 8 hours (hrs) for hatching. Hatched larvae were counted and transferred into properly labeled plastic trays (40 cm x 30 cm x 5 cm) containing 2 L of distilled water [16]. For the purpose of quality control and maintenance of uniform size of the larvae; a density of 1 000 larvae per tray was maintained.

Initially larvae were fed with 1.5 mL of IAEA recommended larval diet. Then the larval diet was added once a day to a tray according to the following regime: day 1, 1.5 mL; day 2, 1.55 mL; day 3, 1.6 mL; day 4, 1.65 mL and day 5, 1.7 mL [15]. Once larvae become pupae (there will be a mixture of larvae and pupae in the tray), pupae were counted and transferred to 500 mL plastic cups containing distilled water using a pasture pipette.

Rearing of the adult Ae. aegypti

Pupal rearing cups were kept inside adult mosquito rearing cages (30 × 30 × 30 cm), until the emergence of adults. After the emergence of adult mosquitoes, they were counted and transferred into an adult rearing cage. Adults were held in (30 cm X 30 cm

X 30 cm) properly labeled cages with a density of seven hundred adult mosquitoes per cage, while maintaining a 1:1 male: female ratio. Adult mosquitoes were fed with 10% sucrose solution. Sugar solutions were replaced every two days to avoid any fungal growth inside the cage. Mosquitoes were reared in an environment with a temperature of 26–27 ^{O}C and 78–80% RH under 12 hrs light and 12 hrs dark cycle [16].

After 3 days since emergence, adult mosquitoes were fed with cattle originated blood. The sucrose solution was removed from adult cage 12–24 hrs prior to feeding with blood meal [17]. For feeding 5 ml of cattle blood was poured into the Hemotek membrane feeder (*PS6, Hemotek*) that maintained the temperature of the blood at 37 °C ± 1 °C, by using a pasteur pipette. The feeder was then placed on top of the adult cage and allowed female mosquitoes to feed for around 1–2 hrs.

Subseuqently, 48 hrs after the blood feeding, egg collection was done by placing an egg collection cup (250 mL) containing 10 mL distilled water, cotton and egg laying filter paper, inside the cage. Egg papers were removed from the cages and left to dry under the standard conditions for 24 hrs. The first instar larvae emerged from above eggs were taken for the experiment by following the standard hatching procedure [17].

Formulation of larval diets

In the current study, efficacy of two different larval diets were compared with IAEA recommended larval diet. The stock solution of the first diet (D_1) was formulated by dissolving dry fish powder meal (25 g) in 100 mL of distilled water. Meanwhile, the second larval Diet (D_2) was comprised of was prepared by dissolving 21.5 g of fish powder meal and 3.5 g brewer's yeast in 100 mL of distilled water. In both diets, the used dry fish powder was purchased from the Peliyagoda fish market (Paliyagoda, Sri Lanka) as a standard product (Fig. 1). The stock slurry of the third larval diet (D_3) was prepared according to the recommended compositions of the International Atomic Energy Agency as the control by dissolving 50% Tuna meal (12.5 g), 36% bovine liver powder (9.0 g), and 14% brewer's yeast (3.5 g) in 100 mL of distilled water [13]. The homogenized stock slurries of three diets were stored at − 20 °C to prevent the degradation of diet components and microbial proliferation. The initial concentrations of the prepared stock slurries were considered as 100% throughout the study.

Larval Feeding Experiment

Three batches of 250 first instar larvae (L_1) of *Ae. aegypti* were counted and transferred in to three separately labelled (D_1, D_2 and D_3) larval rearing trays (25 × 25 × 7 cm) containing 500 mL of deionized water. Each tray was treated with the three larval diets in appropriate volumes, as mentioned above. Fecal matter, and debris in the larval trays were removed daily using pasture pipettes, in order to maintain satisfactory water quality levels for larval development. The papae and adults emerging from three teatments were maintained under standard conditions as described above.

Determination of the life history parameters

Randomly selected larval samples of fourth instar larva (n = 10) and pupa (n = 10) were collected from each diet treatment into the eppendorf tubes with 80% ethanol. In addition, randomly selected adult males (n = 10) and females (n = 10) emerged from each diet treatment were captured from the cages using a mouth aspirator and put into collection vials separately after labelling them. Subsequently, they were killed and preserved by freezing immediately in the refrigerator at 4 ˚C.

Morphometric parameters of the preserved fourth instar *Ae. aegypti* larvae, namely, head length, head width, thoracic length, thoracic width, abdominal length, abdominal width, and total length were measured in the straight position using a digital USB camera fixed in a stereo microscope and OPTIKA version 2.12 image processing software under magnification (1 X) as described in Gunathilaka *et al.* [15]. In addition, the pupation success of *Ae. aegypti* larvae was calculated separately for each larval diet as the percentage of larvae pupated from the total number of introduced larvae. In pupae, the cephalothorax length and width were measured in the straight position using under magnification (1 X). The adult success was determined as the percentage of adults that emerged in relation to the total number of pupae introduced [15].

The right wings of each preserved male and female mosquitoes from separate diet treatments were dissected under a dissecting microscope. Dissected wing and thorax with abdomen were mounted on glass slides separately. The standardized wing length

from the distal alula to the end of the radius excluding fringe scale, and the width of the wing at the greatest breadth excluding fringe were measured under magnification (1 x) [18–19]. In addition, the thoracic length from base of the neck to the base of the abdomen, thorax width from dorsum to the coxae, abdominal length from base of the tip and abdominal width at greatest width were also measured using the digital USB Optica camera mounted on the stereo microscope under magnification (1 X). The number of eggs laid by the females, 2 days after blood feeding was recorded for the three larval diet treatments, separately. In addition, the collected eggs were hatched and the fertility rates were calculated as the percentage of the number of hatched L_1 larvae to the number of eggs laid [16]. Further, the number of days required to eliminate 50% of the male mosqiuotes emerged from different larval diets were reported as male longevity [20].

After 24 hrs since emergence, 100 pupae of one sex were placed in a petri dish (6 cm in diameter and 1.5 cm in height) that open a glass tube (20 cm in height and 7 cm in diameter) and placed in an acrylic cage (30 × 30 × 30 cm). Flight ability was calculated as the percentage of adults that were able to exit from the tube into the cage over a 48 hrs observation period. All of the above described experiments were repeated for three times to maintain the accuracy of the findings.

Statistical analysis

All the data was entered into Microsoft Excel Work Sheets adhering to quality control procedures. IBM SPSS Statistics (version 23 copyright IBM Corporation) was used for data analysis. The effect of different larval diets on morphometric parameters of larvae, pupae and adults was investigated by using the General Linear Model (GLM) followed by Tukey's HSD for mean separation at 5% level of significance. Further, significance in the variations of pupation success, adult success, fecundity, fertility, male longevity and adult flight ability over different larval diets was also analyzed using GLM.

Results

Morphometric parameters of the 4th instar larvae and pupation sucess

The mean growth parameters (head length, head width, thoracic length, thoracic width, abdominal length, and abdominal width) of *Ae. aegypti* 4th instar larvae fed with three different larval feeds are indicated in the Table 1. As the results denote, there was a significant diet-based variation in the head length ($F_{2, 87}$=14.491; *P < 0.001*), head width ($F_{2, 87}$=13.907; *P < 0.001*), thoracic length ($F_{2, 87}$=22.829; *P < 0.001*), thoracic width ($F_{2, 87}$=23.273; *P < 0.001*), abdominal length ($F_{2, 87}$=14.814; *P < 0.001*), abdominal width ($F_{2, 87}$=4.811; *P = 0.01*) and total length ($F_{2, 87}$=7.279; *P = 0.001*) at 95% level of confidence.

Table 1
Morphometric parameters and pupation success of *Ae. aegypti* 4th instar larvae (Mean ± SE) fed with different larval diets

Larval Diet	Head length (mm)	Head width (mm)	Thoracic length (mm)	Thoracic width (mm)	Abdominal length (mm)	Abdominal width (mm)	Total length (mm)	Pupation success (%)
D_1	0.51 ± 0.02[b] (0.49–0.53	0.54 ± 0.02[b] (0.52–0.56)	0.81 ± 0.01[b] (0.80–0.82)	0.94 ± 0.01[b] (0.93–0.95)	3.37 ± 0.04[b] (3.33–3.41)	0.65 ± 0.03[b] (0.61–0.68)	4.71 ± 0.03[c] (4.68–4.74)	78.0 ± 1.5[b] (76.5–79.5)
D_2	0.57 ± 0.01[a] (0.56–0.58)	0.60 ± 0.01[a] (0.59–0.61)	0.88 ± 0.01[a] (0.87–0.89)	1.04 ± 0.01[a] (1.03–1.05)	3.59 ± 0.01[a] (3.58–3.60)	0.70 ± 0.01[a] (0.69–0.71)	4.98 ± 0.04[a] (4.94–5.02)	89.0 ± 2.3[a] (86.7–91.3)
D_3	0.56 ± 0.01[a] (0.55–0.57)	0.59 ± 0.01[a] (0.58–0.60)	0.87 ± 0.01[a] (0.86–0.88)	1.02 ± 0.02[a] (1.00-1.04)	3.50 ± 0.03[a] (3.47–3.53)	0.67 ± 0.02[a, b] (0.65–0.69)	4.85 ± 0.03[b] (4.82–4.88)	83.6 ± 3.4[a] (80.2–87.0)

Note: Values are Mean ± Standard Error (SE) with the range in parenthesis. Different letters in a column denotes significant differences (P<0.05) at 95% level of confidences based on the General Linear Model followed by Tukey's pairwise comparison.

As indicated by the results, the highest values for all the studied growth parameters, namely head length (0.57 ± 0.01 mm), head width (0.60 ± 0.01 mm), thoracic length (0.88 ± 0.01 mm), thoracic width (1.04 ± 0.01 mm), abdominal length (3.59 ± 0.01 mm), abdominal width (0.70 ± 0.01 mm), and total length (4.98 ± 0.04 mm) were observed from the 4th instar larvae fed with the diet 2 (D_2), while the lowest of all the parameters were observed from the larvae fed with the diet 1 (D_1) as indicated in Table 1. Interestingly, head length, head width, thoracic length, thoracic width, abdominal length and abdominal width of the 4th instar larvae of *Ae. aegypti* fed with larval diets 2 (D_2) and 3 (IAEA) belonged to the same cluster, as denoted by the post-hoc analysis of GLM (Table 1).

Meanwhile, pupation success of *Ae. aegypti* larvae fed with different larval diets also denoted significant variations ($F_{2,6}$=20.544; *P = 0.001*) at 95% level of confidence (Table 1). Furthermore the highest pupation success (89.0 ± 2.3%) was observed from the pupae emerged from the larvae fed with D_2 diet, while the lowest was reported from larval diet D_1 as 78.0 ± 1.5% (Table 1).

Impact of the type of larval diet on pupal developmental parameters

Both cephalothoracic length ($F_{2, 87}$=7.803; *P = 0.001*) and width ($F_{2, 87}$=34.181; *P < 0.001*) of the *Ae. aegypti* pupae formed from larvae treated with different larval diets denoted significant differences (P < 0.05) at 95% level of confidence (Fig. 2). The highest cephalothoracic length and width of 1.68 ± 0.01 mm and 2.27 ± 0.02 mm, respectively were observed from the pupae raised from D_2 larval diet. On the otherhand, D_1 diet accounted for the lowest (Fig. 2).

As depicted in Fig. 3, the adult success rates of *Ae. aegypti* pupae reared under different larval diets also denoted a significant variation with the diet ($F_{2, 6}$=11.19; *P = .0066*) at 95% level of confidence. The highest adult success rate of 86.0 ± 1.0% was shown from the larvae fed with D_2 diet, while the lowest (71.0 ± 3.0%) adult success rate was observed from the larvae fed with D_1 diet.

Impact of larval diet on adult developmental parameters

Mean morphometric parameters of adult males and females formed from larvae reared under different larval diets are tabulated in Table 2, along with the results of the GLM. All adult morphometric parameters of adult male and female *Ae. aegypti* mosquitoes varied significantly with different larval diets ($F_{2, 87}$>3.54; P < 0.05 at 95% level of confidence). Comparatively, larger adult males of *Ae. aegypti* mosquitoes emerged from the D_2 larval diet treatment, with highest wing length (5.19 ± 0.03 mm), wing width (1.15 ± 0.02 mm), thoracic length (1.79 ± 0.01 mm), thoracic width (1.38 ± 0.02 mm), abdominal length (4.27 ± 0.04 mm), and abdominal width (0.82 ± 0.04 mm). On the other hand, the smallest male mosquitoes were observed from diet treatment 1 (D_1) as shown in Table 2.

Table 2
Morphometric parameters of *Ae. aegypti* adults fed with different larval diets

Larval Diet	Wing length (mm)		Wing width (mm)		Thoracic length (mm)		Thoracic width (mm)		Abdominal length (mm)		Abdominal width (mm)	
	M	F	M	F	M	F	M	F	M	F	M	F
D_1	5.09 ± 0.03^{b} (5.06–5.12)	5.10 ± 0.02^{c} (5.08–5.12)	1.06 ± 0.02^{b} (1.04–1.08)	1.32 ± 0.02^{a,b} (1.30–1.34)	1.65 ± 0.01^{b} (1.64–1.66)	1.82 ± 0.01^{b} (1.81–1.83)	1.21 ± 0.01^{b} (1.20–1.22)	1.38 ± 0.02^{b} (1.36–1.40)	4.05 ± 0.03^{b} (4.02–4.08)	4.08 ± 0.05^{b} (4.03–4.13)	0.68 ± 0.01^{b} (0.67–0.69)	0.86 ± 0.03^{b} (0.83–0.89)
D_2	5.19 ± 0.03^{a} (5.16–5.22)	5.27 ± 0.04^{a} (5.23–5.31)	1.15 ± 0.02^{a} (1.13–1.17)	1.35 ± 0.02^{a} (1.33–1.37)	1.79 ± 0.01^{a} (1.78–1.80)	1.92 ± 0.02^{a} 1.90–1.94)	1.38 ± 0.02^{a} (1.36–1.40)	1.45 ± 0.02^{a} (1.43–1.47)	4.27 ± 0.04^{a} (4.23–4.31)	4.25 ± 0.05^{a} (4.20–4.30)	0.82 ± 0.04^{a} (0.78–0.86)	0.96 ± 0.03^{a} (0.93–0.99)
D_3	5.11 ± 0.03^{b} (5.08–5.14)	5.19 ± 0.02^{b} (5.17–5.21)	1.14 ± 0.01^{a} (1.13–1.15)	1.30 ± 0.01^{b} (1.29–1.31)	1.78 ± 0.01^{a} (1.77–1.79)	1.88 ± 0.02^{a} (1.86–1.90)	1.35 ± 0.02^{a} (1.33–1.37)	1.43 ± 0.01^{a,b} (1.42–1.44)	4.18 ± 0.03^{c} (4.15–4.21)	4.19 ± 0.04^{a} (4.15–4.23)	0.70 ± 0.02^{b} (0.68–0.72)	0.96 ± 0.02^{a} (0.92–0.96)

Note: Values are Mean ± Standard Error (SE) with the range in parenthesis. Different letters in a column denotes significant differences (P<0.05) at 95% level of confidences based on the General Linear Model followed by Tukey's pairwise comparison.

Similar to males, the highest wing length (5.27 ± 0.04 mm), highest wing width (1.35 ± 0.02 mm), highest thoracic length (1.92 ± 0.02 mm), highest thoracic width (1.45 ± 0.02 mm), highest abdominal length (4.25 ± 0.05 mm) and highest abdominal width (0.96 ± 0.03 mm) were observed from the adult females emerged from the D_2 diet treatment, while the lowest growth parameters were observed from the adult females emerged from the diet treatment D_1, except for wing width (Table 2).

Impact of the type of larval diet on behavior and biology of Ae. aegypti

Fecundity (egg production per 100 fed female) was significantly affected by different larval diets ($F_{2, 6}$= 8.294; P = 0.014 at 95% level of confidence) provided during the larval stage based on the statistics of GLM (Table 3). The highest egg production (1453.4 ± 23.5) was observed from the females formed from larvae reared under diet 2 (D_2), while the lowest egg production (1364.2 ± 12.8) was observed from the females of D_1 diet. However, the hatching rate/ fertility was not significantly influenced by different larval diets given at the larval stages of *Ae. aegypti* ($F_{2, 6}$= 4.397; P = 0.057) at 95% confidence level (Table 3). Fertility rates of eggs produced by female mosquitoes of larval diets D_2 (98.0 ± 0.3%) and D_3 (97.5 ± 0.5%) remained relatively higher, while larval diet D_1 reported the lowest hatching rate.

Table 3
Life history parameters of *Ae.aegypti* fed with different larval diets

Larval Diet	Fecundity	Fertility (%)	Survival time/ Longevity (days)	Flight ability (%)
D1	1364.2 ± 12.8^{b} (1351.4–1377.0)	95.4 ± 0.6^{a} (94.8–96.0)	15.5 ± 0.7^{b} (14.8–16.2)	96.0 ± 0.5^{a} (95.5–96.5)
D2	1453.4 ± 23.5^{a} (1429.9 ± 1476.9)	98.0 ± 0.3^{a} (97.7–98.3)	18.2 ± 0.4^{a} (17.8–18.6)	98.3 ± 0.2^{a} (98.1–98.5)
D3	1441.0 ± 20.1^{a} (1420.9-1461.1)	97.5 ± 0.5^{a} (97.0–98.0)	18.0 ± 0.3^{a} (17.7–18.3)	98.2 ± 0.2^{a} (98.0-98.4)

Note: Values are Mean ± Standard Error (SE) with the range in parenthesis. Different letters in a column denotes significant differences (P<0.05) at 95% level of confidences based on the General Linear Model followed by Tukey's pairwise comparison.

At 95% level of confidence, there was a significant variation in the survival time (longevity) of the male adult mosquitoes ($F_{2, 6}$= 7.897, P = 0.016) as shown in Table 3. The longest survival time (18.2 ± 0.4 days) was observed from the males emerged from the D_2 larval diet treatment and the lowest survival time (16.0 ± 0.7 days) was observed from the adults emerged from the D_1 larval diet treatment. Further, eventhough the flight ability of adult mosquitoes did not show any diet based variations ($F2_{, 6}$= 21.427; P = 0.074), the adults fed with diet 2 (D_2) and IAEA diet (D_3) denoted relatively higher flight abilities (> 98.2 ± 0.2%) as indicated in Table 3.

Discussion

Success of novel biological control strategies such as SIT and IIT programs depend upon mass production of good quality sterile insects to be released into target areas. For this, a balanced larval diet, that can ensure high survivorship, fast and homogeneous larval development, uniformity in body size and production of healthy high quality males, is desired. Use of a nutrious, yet cost effective larval diet, is a key factor, which can influence the above aspects in mass rearing of *Aedes* vectors. IAEA diet is the larval diet recommended and currently used in many countries for mass rearing of *Ae. aegypti*. However, local production of it is costly and challenging due to practical difficulties concerning the availability of the bovine liver powder component, to ensure affordability and sustainability of mosquito production [21]. Therefore, the current study aimed to develop an alternative and economic diet mixture from locally available materials for mass rearing purposes, while ensuring optimum quality of *Ae. aegypti* mosquitoes.

Conditions faced during the larval stage of mosquitoes could play a crucial role in population size regulation. These factors may be either density-dependent or independent [22]. Among those, factors such as water temperature, water depth of the container, food quality and quantity have been identified to cause significant impacts on the larval stages [23]. The larval diet of *Ae. aegypti* could limit their larval growth rate, survival period, and size [24–25]. According to Gilles *et al*. [26], increasing larval density at lower diet levels could prolong the development time, while higher food levels tend to shorten the time to pupation. All the environmental conditions in the containers (except for larval diet level) were uniformly maintained in this study to specifically investigate the effects of studied larval diets on different morphological and behavioural parameters of *Ae. aegypti*.

In the current study, *Aedes* larvae showed a significant difference in their head length, head width, thoracic length and thoracic width due to differnt diet treatments. Only the abdominal length, abdominal width and the total length did not show any significant difference with the diet treatment. In all these parameters, the highest growth level was observed from the larvae treated with the experimental diet D_2 and IAEA recommended diet, while the least growth was observed in D_1. This reveals that there is a significant effect of adding yeast to the larval diet mixture to enhance the growth of *Ae. aegypti* larvae. Yeast have less protein and higher content of carbohydrates. Therefore, *Ae. aegypti* larvae can store and utilize their energetic components efficiently [27].

According to literature, the duration of adult mosquito emergence from the L_1 larval stage, pupation and adult success levels become faster when all nutrients are abundant [28]. Thus, results of the current study evidence that both D_2 and IAEA diets comprise of all the nutritional components (sugar and other digestible carbohydrates) required for the larval development. Carbohydrate amount consumed in the larval stages, is directly associated with pupation [29–30]. Therefore, the significantly lower pupation success rate shown in D_1 diet may be due to the limited sugar availability, resulting in a developmental delay. As the cephalothoracic length and cephalothoracic width denoted significant variations with the larval diet, those parameters can be used as reliable parameters for predicting adult male body size in SIT or IIT programmes.

Generally, the males tend to emerge before females under both natural and laboratory conditions. Any scarcity or excess amount of food could preferentially influence the proportions and survival of females over males [31]. The results of the present study remain in line with literature, evidencing that male mosquitoes take less time to attain adulthood and survive longer [32]. Therefore, males tend to withstand starvation conditions more than the females during the larval stage and have a lower nutritional threshold for pupation than females [33]. These traits might have influenced the quicker developmental rates observed in males and could have increased their survival.

Body size is more important for females as it influences a variety of factors including the fecundity during their lifetime [34], level of dispersion, host attack rate [35], and the frequency of blood meals required to survive [36]. Nutrient reserves of adults (mainly

glycogen and triglycerides) obtained during the larval stage could extend the longevity of adult mosquitoes [37]. The significantly shorter longevity of adult males emerged from the larvae supplied with D_1 diet could be explained by this fact, signifying the lack of glycogen and triglycerides in sufficient amounts required for the successful larval growth, unlike the D_2 and IAEA diets.

Carbohydrates and lipids also influence the flight ability of adults. Results of the present study indicate that the experimental diet D_2 and IAEA recommended diets are containing higher levels of carbohydrates and lipids, as both longevity and the flight ability were significantly similar. A similarly higher longevity of males have been observed by Bond *et al*. [9], when using a Laboratory Rodent Diet (LRD) as a larval diet. Studies on longevity of adult males with different diet samples emphasize that the energy reserves synthesized and accumulated in the larval stages have important consequences on the longevity of adults [38]. Prolonged survival of male mosquitoes is a critical requirement for the success of IIT and SIT programmes [39]. In the current study, the experimental diet D_2 performed better than the IAEA and D_1 diets, in terms of adult male longevity.

Larval nutrient reserves (protein, lipid, and glycogen) are also important for egg production and the endocrine regulation of egg development in *Ae. aegypti* [40]. High levels of glycogen and protein can induce the ovarian ecdysteroid production in females and inhibit juvenile hormone biosynthesis by the corpora allata, resulting enhanced vitellogenesis and egg production [40]. On the other hand, few studies have reported that lipid reserves influence pupal commitment, and the endocrine regulation of egg development in autogenous and anautogenous female mosquitoes [25;40]. The current study reported a significantly higher edd production rate from the larvae reared under D_2 diet, suggesting that the newly formulated diet is acceptable interms of fecundity. The eggs of the females that developed under the D_2 diet had a relatively higher fertility than females from the IAEA and D1 diets, further supporting the above claim. A similar fining has also been reported from a LRD, suggesting that the D_2 diet in the current study is acceptable interms of carbohydrate and energetic content [9].

The selection of a proper diet should be done after considering the feeding pattern and feeding regime of the considering mosquito species that will be mass cultured. *Ae. aegypti* has been identified as a mosquito species, which are adapted to nutrient poor-habitats, as food limitation is a major regulator of their population size [33]. The *Ae. aegypti* development rate decreases in response to food scarcity, until reaching a critical threshold of nutritional reserves [12]. Thus, the ability to survive periods of limited food is a critical larval fitness parameter [33]. The experimental D_2 diet caters well for this requirement by performing similar or better than the IAEA recomanded diet, which is being widely used.

The IAEA diet, which is being used as a standard larval diet in many countries, includes tuna meal, bovine liver powder (BLP) and brewer's yeast, which are rich in proteins, vitamins, and fatty acids [13, 41]. However, mass rearing of *Ae. aegypti* requires a nutrient rich, economic and readily available diet that can be stored for a considerable time. The evaluated experimental diets were comprised of accessible ingredients, namely dry fish powder obtained from the fish market and yeast. Since BLP is not included, the production cost of the novel diets are significantly less and dry fish powder component is readily available as an animal feed at the fish market. Regardless of the absence of BLP and tuna meal, the experimental diets include discarded scales, skeletons of fish and shellfish such as crustacean and mollusk, which are also rich in proteins [42]. Therefore, dried fish powder act as a protein reservoir and replace the effect of BLP in the diet.

According to Reisen [43] overfeeding of larvae can often lead to high larval mortality. Gilles *et al*. [26] has revealed that, excessive larval diet can reduce the larval survival due to microorganisms, which proliferate unconsumed food availabile in the medium. And also, the quantity of diet added in the larval tray has a great influence on the growth and development of larvae [41]. Therefore the amount of diet provided to mosquito larvae is clearly a crucial parameter to take into account, and in this study all the larval diets were added following the standard regime practiced for IAEA diet.

Based on the performance of experimental D_2 diet, interms of larval, pupal and adult life developmental parameters, functional and behavioral parameters, it is clear that D_2 diet is an economic substitute for the IAEA diet. The IAEA recommended diet has been estimated to cost approximately USD 64.26 per kilogram [13], while the D_2 experimental diet costs only USD 2.32. Therefore, the present study reveals that the experimental D_2 diet has a similar nutritional value to IAEA diet ensuring the optimal development of *Ae. aegypti* larvae and production efficiency, making it a better larval supplement to be used in mass rearing of *Ae. aegypti*.

Conclusion

Findings of the current study revelaed significant diet-based variations in *Ae. aegypti* larvae in terms of all the morphometric parameters at 95% level of confidence. The significantly largest larvae were produced from the D_2 experimental diet, which also denoted the highest pupation success rate also. Further, the cephalothoracic length and width also denoted significant differences among the dietary treatments, suggesting that D_2 diet results the highest quality pupae interms of morphometry and pupation sucess. All adult morphometric parameters of adult male and female *Ae. aegypti* mosquitoes also varied significantly with different larval diets ($P < 0.001$). In addition, significantly higher fecundity and male longevity levels were shown by the adult *Ae. aegypti* ($P < 0.05$) reared under diet D_2. As a whole, the larval diet D_2 performed significantly better or equally to the IAEA diet, suggesting that the new formulation is acceptable interms of nutirion and energetic componets. Since, D_2 diet is derived with readily available local ingredients that are inexpensive, it can be recomanded for the mass rearing of *Ae. aegypti* for area-wide IIT and SIT-based vector control in Sri Lanka.

Declarations

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Authors' contributions

US conducted laboratory experiments and wrote the manuscript. LU designed the research, conducted statistical analysis and wrote the manuscript. MG supervised the research work and reviewed the manuscript. MH supervised the research work and reviewed the manuscript. TR designed the research, supervised the research, conducted laboratory experiments and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Ethics approval and consent to participate

Ethical clearance for the study was obtained from Ethical Review Committee (ERC) (8/2019) of the Faculty of Medicine, University of Kelaniya, Sri Lanka.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

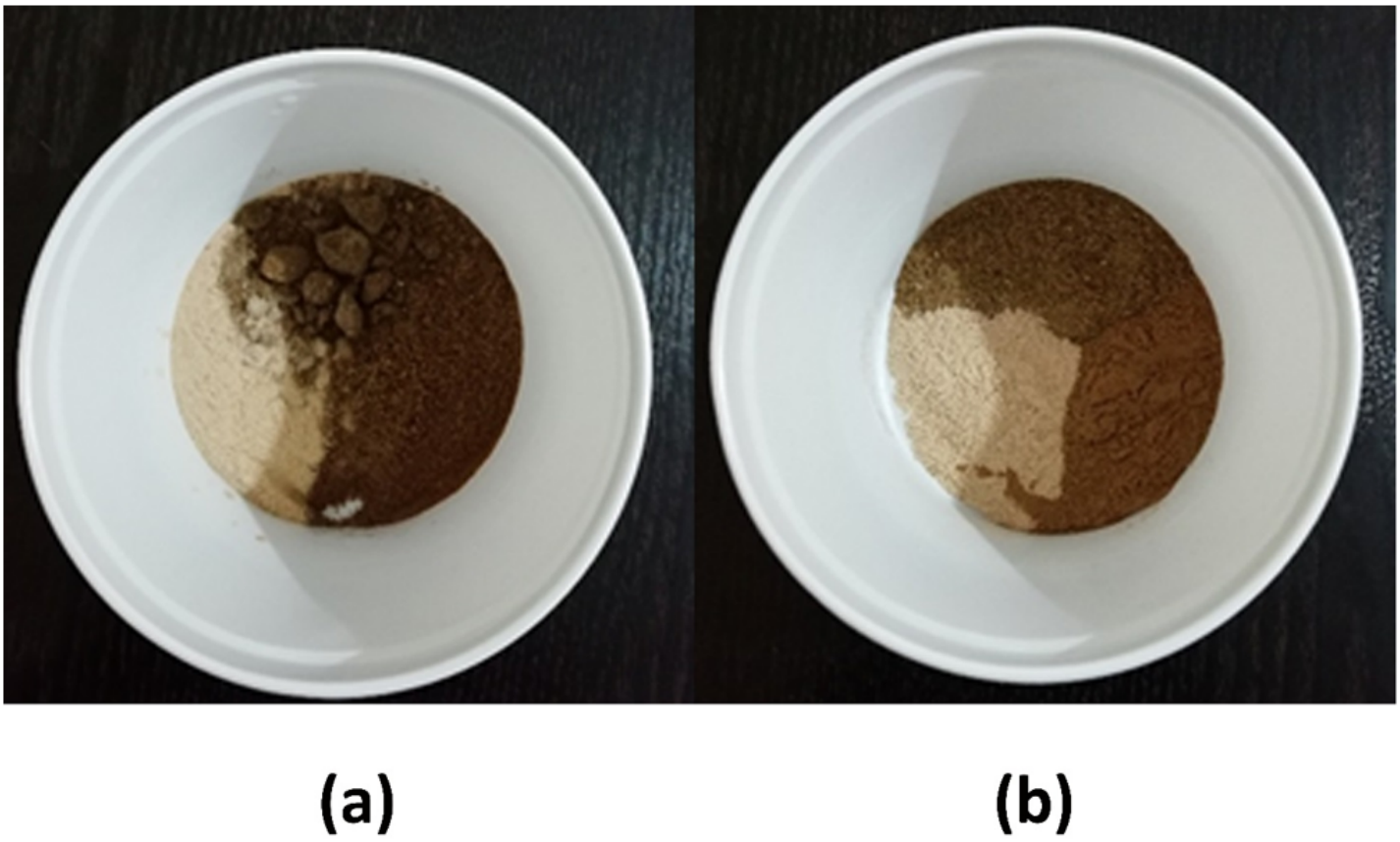

Figure 1

(a) dried fish powder and (b) IAEA diet mixture used for the preparation of the larval diets

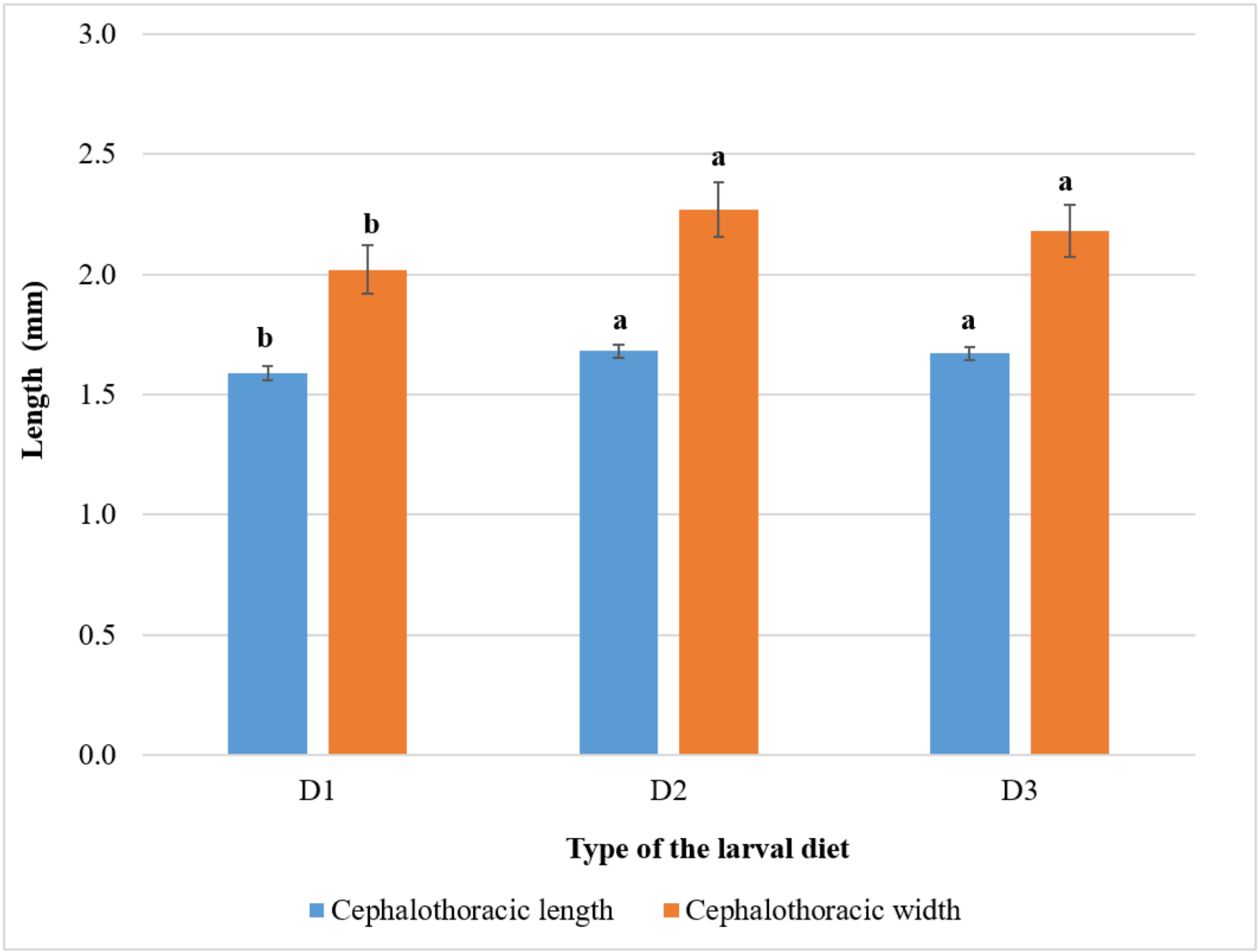

Figure 2

Mean cephalothoracic length and width of the Ae. aegypti pupae formed from larvae treated with different larval diets. Different letters in over columns denote significant differences (P<0.05) at 95% level of confidences based on the General Linear Model followed by Tukey's pairwise comparison.

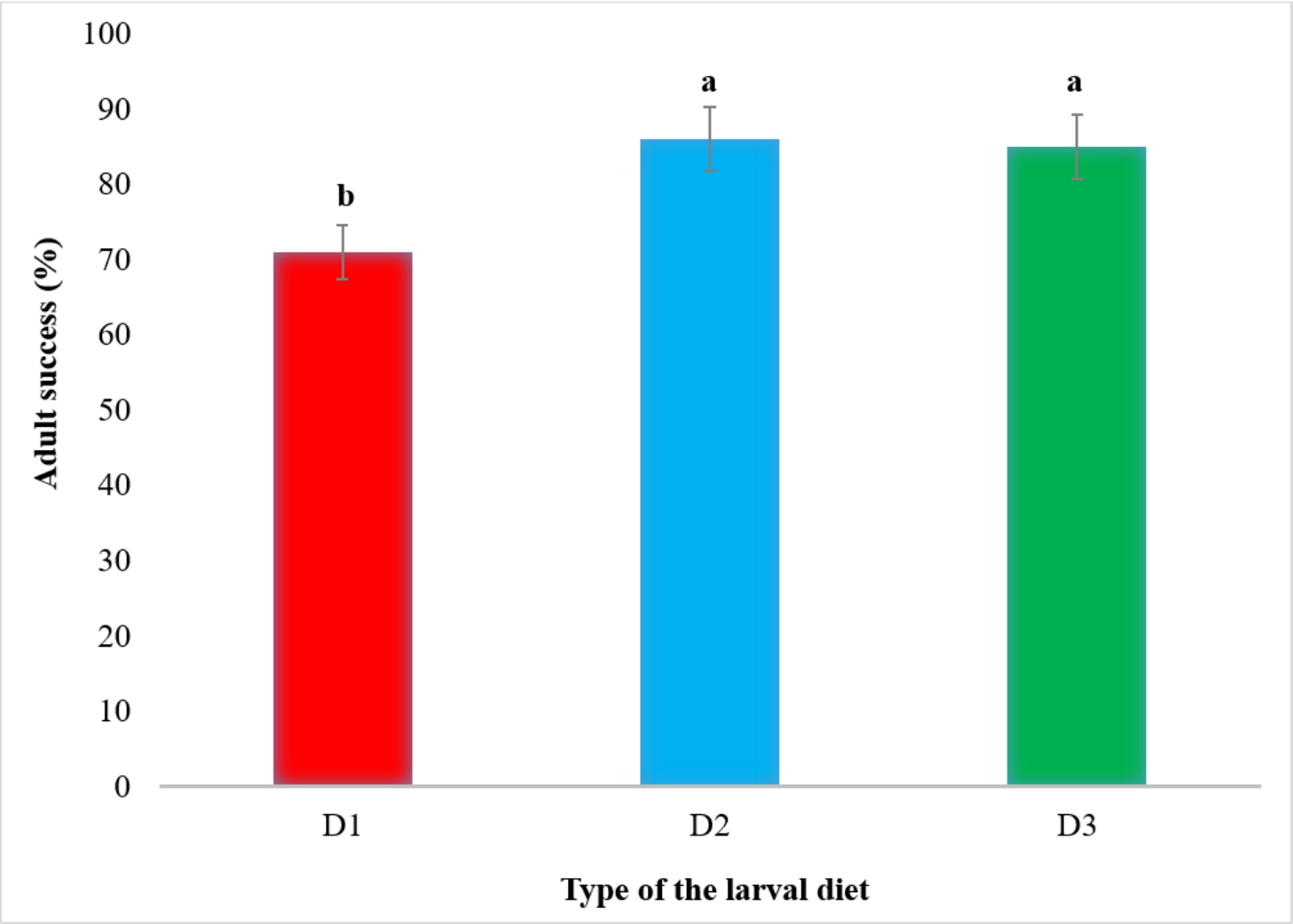

Figure 3

Mean adult success rates of the Ae. aegypti larvae reared under different larval diets Different letters over columns denote significant differences (P<0.05) at 95% level of confidences based on the General Linear Model followed by Tukey's pairwise comparison.