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Efficiency of Two Larval Diets for Mass Rearing of *Aedes aegypti* in Support of Sterile Insect Technique (SIT)

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Abstract

Aedes aegypti is a major vector of arboviruses such as dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV) that may be controlled on an area-wide basis using the sterile insect technique (SIT). Larval diet is a major factor in mass-rearing for SIT programs. Accordingly, we compared dietary effects on immature development and adult fitness-related characteristics for laboratory based fish feed diet 'Super Nova' (Super Nova, Perfect Companion Group Co. Ltd., Thailand) and high quality feed for fingerlings, 'Micro-180' (Skretting, Neutreco company, USA) to use in SIT. The percentage of larvae that survived to pupation or to adult emergence did not differ significantly between the two diets under the same rearing conditions. On the second day of development, a good number of male pupae (69-71% among total male pupae) produced from both the diet treatments. The flight ability and the longevity of *Ae. aegypti* did not differ significantly with larval diets, irrespective of sex. In other aspects, the 'Micro-180' diet generally performed better. Larval development was faster on the 'Micro-180' than 'Super Nova' fish feed diet. An Adult female mosquito from the 'Micro-180' diet was significantly larger and exhibited higher fecundity compared to 'Super Nova' fish feed diet. We concluded that both the diet treatments are suitable and especially the 'Micro-180' diet is likely to be more suitable for mass-rearing of *Ae. aegypti* for area-wide SIT-based vector control.

Keywords: *Aedes aegypti*, larval diets, mass rearing, quality parameters, SIT

Introduction

Aedes aegypti is the most widespread and potential vector for transmission of dengue disease [1] and this mosquito species is also responsible for spreading other vector-borne diseases like chikungunya, zika, malaria, dengue fever, encephalitis, yellow fever and filariasis [2,3]. Worldwide *Ae. aegypti* species has been identified as one of the highest invasive species [4,5]. For worldwide distribution of *Ae. aegypti*, around 2.5 billion people are at threat of dengue infection and every year approximately 50 million people are infected by dengue [1]. *Ae. aegypti* is well-known as a container-breeding mosquito species because it completes its aquatic immature life stage (egg to pupa) in artificial water containers or stagnant water containing natural sites of urban and suburban areas throughout the world [6,7]. They are exceedingly anthropophilic potential vectors and usually active at the diurnal time. The prevention of the transmission of the dengue virus can be achieved by the reduction of *Ae. aegypti* population because the virus transmission mostly depends on the *Ae. aegypti* vector.

The main tactic to reduce mosquito population is habitat eradication and mosquito breeding habitats can be treated with different chemical insecticides like larvicides and adulticides as components of the integrated way to manage mosquito population [8-10]. Therefore, chemical insecticides act as a potential and highly practiced way of control against *Ae. aegypti*. However, it is not only difficult but also quite impossible to eliminate all the breeding sites of *Ae. aegypti*. Moreover, overexposure to insecticides is associated with harmful effects on non-target

organisms of environments and mosquito populations be-
come resistant to conventional insecticides [11]. Hence, the
development of novel, innovative and effective approaches
for the control of dengue vectors are immediately required
to overcome the present challenge, with exact importance
on methods that are environmentally friendly, cost-effective,
suitable and sustainable for the integrated pest management
programs [12,13]. Over the last few decades the Sterile Insect
Technique (SIT) as a major component of area-wide integrated
pest management (AW-IPM) has regained interest as a com-
plementary tool to the current mosquito control methods to
limit the transmission of mosquito-transmitted pathogens
[14-17]. The SIT represents the most promising, environmen-
tally friendly and sustainable way of mosquito control rather
than other different traditional and conventional strategies.
SIT is a species-specific suppressing biological method that
crucially depends on mass rearing, sterilization, and release
of large numbers of sterile males [18]. The consequence of
releasing a huge number of sterile males to an area (which
is mainly specific and separated from any other area by
naturally occurring physical barrier) decreases the mosquito
population level through competing wild males to mate
with wild females and introduce sterility [19,20]. Effective
control of a devastating insect population can be achieved
by the systematic management of sterile insects in an AW-
IPM program [21,22].

However, larval diet is considered as one of the most
critical and costly components of the operational running
costs, in the context of applying the SIT [23]. The quality of
larval diets has a direct and irreversible influence on adult
qualities [24,25], mainly on the body size of the laboratory-
reared adults, and other biological functions related to their
nutrition. The previous study reported that well-fed larvae
become healthy and vigorous adults [26]. Quantitative and
qualitative parameters of larval nutrition present significant
consequences on immature longevity and development
rate, which can modify population growth of mosquitoes
and fix adults characteristics [27-29]. Hence, it is important
to ensure that the larval diet should contain rich nutrients to
escape the risk of deficiencies that might deleteriously dis-
turb the developmental period, the rearing productivity and
the vigor of the adult males produced [30]. For a successful
SIT program it is important that the larval diet leads to high
rearing productivity and subsequent quality of the adults
produced. If the laboratory-reared adults are not compat-
ible with the existing wild population, projected results of
the SIT programs would not achieve and develop. The BLP
(Bovine Liver Protein) in the IAEA standard diet worked well
for the rearing of *Ae. aegypti*, but this is a costly ingredient
and difficult to procure to many FAO and IAEA member states
[31], especially in Bangladesh. Therefore, the search for other
sources of larval diets and their effects on different quality
parameters of mosquitoes should need to further evaluated
for mass rearing of *Ae. aegypti* targeting SIT application.

The present study was therefore undertaken to evaluate the
efficiency of the two different artificial larval diets regard-
ing their effects on larval development and different quality
parameters (*viz,* pupal yield (number), sex ratio, flight ability,
mating capability, fecundity, percentage of egg hatch, and
longevity) of *Ae. aegypti* under laboratory conditions. A com-
mercial aquarium fish feed (Super Nova, Perfect Companion
Group Co., Ltd) that has been regularly used to rear *Ae. aegypti*
in our laboratory, and a high quality fingerling fish feed 'Mi-
cro-180' (Skretting, Neutreco company, USA) was used for this
purpose. Experimental findings were discussed in the context
of using mass reared *Ae. aegypti* for the application of SIT.

Materials and methods
Mosquito culture
The research experiment was conducted in Insect Biotechnol-
ogy Division (IBD), Institute of Food and Radiation Biology
(IFRB), Atomic Energy Research Establishment (AERE), Savar,
Dhaka. The laboratory colony of *Ae. aegypti* strain was reared
under controlled conditions at 27 ± 1℃, 70 ± 10% relative
humidity (RH), and photoperiod of 12 h light (L):12 h dark (D).
To compare the effect of two larval diets on *Ae. aegypti*, 1000
first-instar (3 hours old) larvae were picked up and transferred
to plastic rearing trays (38 x 26 x 1.5 cm) containing 650 ml
of water to ensure a density of 1.5 larvae per ml of water for
maintaining optimum condition for mass-rearing of *Ae. aegypti*
(0.6±2.8 larvae/ml) [32]. Each diet was supplied (0.7 mg diet
per larva) to tray on each day up to pupation. Three replicates
were maintained for each diet treatment and each parameter.
All trays were checked three times a day and replaced the
water if water level down due to evaporation. Larvae that
turned into pupae were collected by a 3 ml plastic pipette,
recorded and placed in water containing 50 ml beakers. The
sex of pupae was manually separated according to their size
(female pupae larger than male pupae). Adult mosquitoes
emerged approximately after two to three days of pupation.

Diets
Two different types of fish feed used in the present study.
'Diet-1' was a high quality fingerling feed 'Micro-180' (Skretting,
Neutreco company, USA). Our laboratory used commercial
aquarium fish feed (Super Nova, Perfect Companion Group
Co. Ltd., Thailand) was used as 'Diet-2'. Diet 1 (Micro-180)
comprised of crude protein 38.0%, crude fat 6.0%, crude
ash 11.0%, crude fiber 3.0% and crude moisture 11.0%. The
ingredients of 'Diet 2' consisted of fish meal, wheat grain,
yellow corn, rice bran, soybean meal, vitamins, minerals, food
colouring, and antioxidants with protein 20.0%, fat 3.0%, fiber
7.0%, moisture 10.0%, calcium 0.7%, phosphorus 0.7%.

Quality parameter evaluations
The quality parameters of *Ae. aegypti* reared on two different
diet treatments were evaluated by assessing larval duration
(days), pupal yield (number), sex ratio, flight ability, mating

capability, fecundity, percentage of egg hatching, and survival. All quality parameters were estimated and performed under the controlled laboratory condition (27±1℃, 70 ± 10% RH, and photoperiod of 12 h L:12 h D).

Larval development and pupal yield
Total larval period/time to pupation and time to adult emergence were calculated according to the duration of the development from the first instar larvae until the pupal formation. Survival to pupation and survival to adult emergence was determined according to the proportion of pupae that survived in relation to the total number of larvae placed in each tray. The production of male and female pupae were recorded in the duration of total pupation time in each diet treatment. The overall sex ratio was calculated as the total number of adult males in relation to the total number of adult females that emerged in each diet treatment.

Body size
Adult male and female *Ae. aegypti* developed from each diet treatments were stored at the deep freeze, and the size of the adults was estimated by measuring their right-wing from the distal edge of the alula to the end of the radius vein excluding fringe scales, which is a reliable indicator of body size [33]. Wings of adults were measured under-stereo microscopes. In total, 10 adults were selected for each diet to measure wing length.

Flight ability
To determine the flight ability of adults, 100 pupae were placed into a 50ml beaker which was introduced into tubes (30cm H x 6cm W) and placed in a small cage (33 x 26 x 20 cm). Flight ability was measured according to the prevalence of adults that emerged from the pupae and were able to exit from the tube into the cage over a 48 hours period. This test was performed three times for the pupae of both diets treatments.

Mating capacity
For this purpose, 100 females and 100 males developed from both the diet treatments were separately placed inside the cages (33 x 26 x 20 cm). The mating behaviour was observed directly inside the cages over a period of two hours and the formation of a number of mating pairs was recorded. This experiment was repeated three times.

Fecundity and fertility
Males and females (100 for each) *Ae. aegypti* developed from each diet treatment were placed inside small cages (33 x 26 x 20 cm) and supplied 10% (wt/vol) sugar solution. Poultry blood was supplied using a membrane feeding system after three days. At 24 hours after the first blood meal, a 250 ml beaker was placed in each cage with water and a strip of white paper as an oviposition substrate. The white paper strip was removed daily and replaced by another. Eggs were

counted and the fertility was measured by determining the percentage of hatching.

Adult longevity
To determine the longevity of males of each diet treatment newly emerged 100 males were selected at random, placed in a small cage (33 x 26 x 20 cm) and supplied with 10% sugar solution. Separate batches of mosquitoes from both the diet treatments were also kept with only a water supply. Each treatment was replicated three times. All dead males found each day in each replicate were counted and removed at 24 hours interval. Adult mortality was recorded daily until the last adult had died.

Statistical analysis
Data collected for larval development and quality parameters of *Ae. aegypti* on both the diet treatments were analyzed using Analysis of Variance (ANOVA) using Statistical Software –Minitab, USA (version-17). The graph was created in Microsoft Excel 2007.

Results
Quality parameter
The quality parameters of *Ae. aegypti* reared on two different diet treatments were shown in **Table 1** and **Figures 1**-**4**.

Figure 1. Mean (± se) pupal production of *Ae. aegypti* reared on two different larval diets.

Figure 2. Total number of male and female pupal production of *Ae. aegypti* per day reared on two different larval diets.
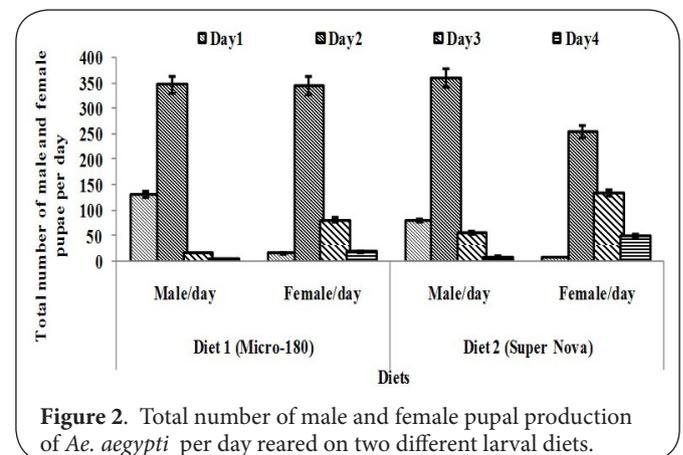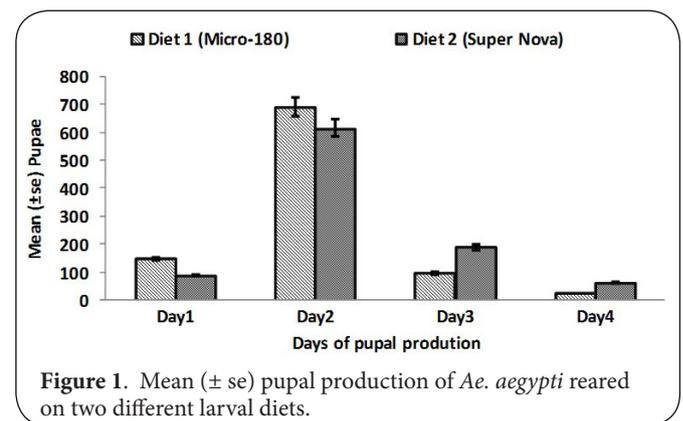
Table 1. Quality parameters of *Ae. aegypti* reared on two different larval diets.

Quality Parameters	Diet 1 (Micro-180)	Diet 2 (Super Nova)	Significance (ANOVA)
Total pupal production/pupal yield (number)	961.5±152.82a	953.5±128.77a	d=1,6; F=0.00; P= 0.992
Larval developmental time/Time to pupation (days)	4.33±0.33a	5.5 ± 0.28b	d=1,4; F=7.00; P=0.057
Adult emergence (%)	97.66±0.66a	98.33±0.33a	d=1,4; F=0.80; P=0.422
Sex (% male)	52.00a	53.01a	d=1,4; F=1.59; P=0.276
Flight ability (%)	96.0 ± 0.28a	95.1 ± 0.44a	d=1,4; F=2.50; P=0.189
Mating capacity (number of pairs)	87.66±2.90a	91.66±1.33a	d=1, 4; F=1.57 ; P=0.279
Hatching (%)	70.33±0.1a	69.33±0.5a	d=1, 4; F=0.64 ; P=0.468
Longevity (days)	33.00 ±0.57a	34.16 ± 0.44a	d=1,4; F=2.58; P=0.184

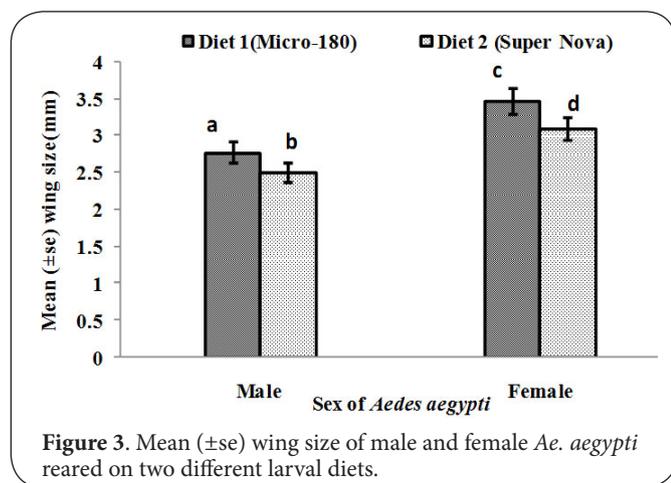*Means followed by the same letter are not significantly different (P>0.05).

Figure 3. Mean (±se) wing size of male and female *Ae. aegypti* reared on two different larval diets.
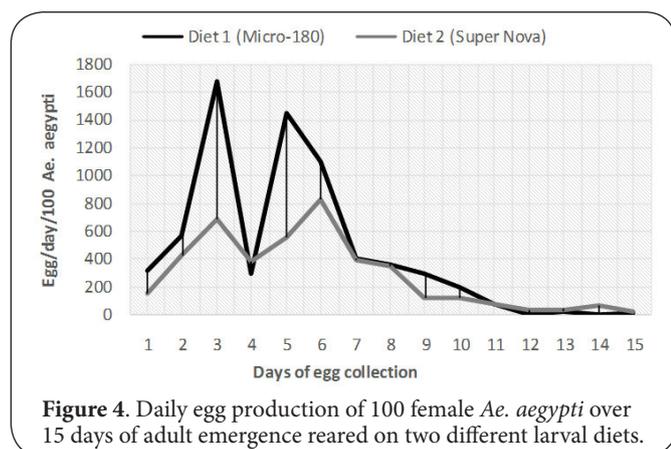

Figure 4. Daily egg production of 100 female *Ae. aegypti* over 15 days of adult emergence reared on two different larval diets.

Development time

Comparatively faster larval development of *A. aegypti* i.e., first-instar larvae to first instar pupae was recorded on Diet 1 (Micro-180 diet) (mean 4.33±0.33 days) than Diet 2 (Super Nova) (5.5±0.28 days) which differed significantly (d=1,4; F=7.00; P=0.05). However, no significant differences were observed in first-instar larval stage to pupal development

times between the male and female pupae on either diet. The highest number of male pupae (69-71% among total male pupae) produced on the second day of development from both the diet treatments. Time from pupae to adult emergence (2-3 days) did not differ between the diet treatments.

Production of male and female pupae

The total pupae produced from Diet 1 and Diet 2 did not differ significantly (d=1,6; F=0.00; P=0.99) (**Figure 1**). Percentage of total pupal production was 96.50% and 95.35%, respectively from Diet 1 (Micro-180 diet) and Diet 2 (Super Nova). Male and female pupation rate of *Ae. aegypti* reared on Diet 1 diet was 52.00% and 48%, respectively. It was 53.01% and 46.99%, respectively for those developed from Diet 2 over 4 days of total pupal production.

Comparatively more male pupae (131.5±38.5) developed on the first day on Diet 1 than on Diet 2 (80.5±31.5). But an almost similar number of male pupae developed from both the diets on the second day (347.5±71.5 and 360.5±99.5, respectively). Comparatively greater number of females produced on the second day on Diet 1 (345±33) than Diet 2 (255 ±49) (**Figure 2**). Maximum number of male and female larvae pupates on the second day of development from both the diets.

Body size (wing length)

Mean wing length of males *Ae. aegypti* differed significantly with larval diets (df=1,18; F=23.35; P=0.000). Male wing size recorded as 2.7±0.03mm from those developed from Diet 1, and it was 2.5±0.03mm for males of Diet 2. Significantly (df=1,18; F=13.13; P=0.002) longer wing length was also recorded for females (3.47±0.07) developed from Diet 1 diets than those of Diet 2 (3.09±0.07 mm) (**Figure 3**).

Flight ability and mating capacity

The flight ability of *Ae. agepti* developed from Diet 2 was 95.1 ± 0.44%. It was similar to those of mosquitoes emerged from the Diet 1 (96.0 ± 0.28%) and did not differ significantly (d=1,4; F=2.50; P=0.189). Comparatively less number of copulations (87.66±2.90) was recorded for mosquitoes developed from

Diet 1 than those reared on Diet 2 (91.66±1.33) under laboratory condition although differences were not significant (d=1, 4; F=1.57 ; P=0.279).

Fecundity and fertility
The fecundity of females was measured in terms of egg production from each cage of 100 female + 100 male mosquitoes over the first 15 days of adult emergence (**Figure 4**). The fecundity of females from the Diet 1 was ~6790 eggs; 4.52 eggs/female/day) higher than females from the Diet 2 (4217; 2.81 eggs/female/day). However, the difference was not significant (d=1, 28; F=1.26; P=0.272). The fertility of eggs laid by females, expressed as the percentage of egg hatching, was ~70% in both the diets, i.e., Diet 1 and Diet 2.

Adult longevity
The longevity of adult males maintained with water alone did not differ between diet treatments and averaged between 7.81±1.55 and 7.25±0.03days. The longevity of adult males that were caged together with females with access to sugar solution also did not differ significantly between diet treatments (d=1,4 F=2.58; P=0.184) with mean longevity values of 33.00±0.57 days, and 34.16±0.44 days on Diet 1 and Diet 2 respectively, under laboratory condition.

Discussion
A series of laboratory studies revealed that *Ae. aegypti* reared on Diet 1 (Micro-180) have a shorter larval developmental time, produced larger adults and greater fecundity than those developed from Diet 2 (Super Nova) (**Figures 3** and **4**). Generally a short pupation time and pupal development period prior to adult emergence are desirable in a mass rearing program, as these increase the overall rate of insect production. The timing of metamorphosis in *Ae. aegypti* larvae are known to be influenced by both the availability of food and temperature [**34**]. Nutritional reserves, particularly glycogen, play a regulatory role in insect development influencing the ability of larvae to pupate. In the present experiment, the pupation time from the 1st instar larval stage was shorter in *Ae. aegypti* reared on Diet 1 compared to Diet 2 may reflect this statement. According to Mamai *et al.,* [**31**] total pupation of *A. aegypti* took 7.99 ± 0.06 days in the IAEA diet which consists of 50% TM + 35% BLP + 15% BY as well as in two other diets they examined using two different formulations of Black soldier fly (*Hermetia illucens* L.) larvae powder. Whereas in the present study total pupation of *Ae. aegypti* completed within 4.33±0.33 days using Micro-180 diet under laboratory conditions. Although longer developmental time was recorded on Diet 2 (5.5 ± 0.28 days). The present findings have some similarities with the observation of Bond *et al.,* [**35**] who reported *Ae. aegypti* larvae fed with IAEA (IAEA 2) diet (consisted of 25% (wt/wt) bovine liver powder, 50% tuna meal, and 12.5% brewer's yeast) in dark conditions developed significantly faster (mean 3.78 days) than larvae on the Laboratory Rodent Diet (LRD) Lab Diet

5001 (PMI Nutrition International LCC, St. Louis, MO) under dark conditions (4.14 days). In the present study formation of male pupae were a bit earlier than the female pupae in both diets as recorded by Mamai *et al.,* [**31**] in the case of *Ae. aegypti* mentioned earlier.

Usually, body size reflects nutritional status, and the level of teneral reserves in female mosquitoes is known to affect their body size, fecundity, longevity and blood meal consumption and utilization [**36,37**]. Comparatively bigger body size was observed for both the male and female *Ae. aegypti* reared on Diet 1 (Micro-180) than those developed from Diet 2 (Super Nova). The differences in the size of females are likely related to the greater energy content (comparatively higher carbohydrate 6%) of the Diet 1 (Micro-180) since energy intake during the larval stage has a direct influence on the adult size in this species. It has been reported that adult teneral reserves play a vital role in the pre-vitellogenic phase of ovarian development of an autogenous mosquito [**38**], females use reserves carried over from the larval stage to develop the first batch of eggs. Usually, the large females are more likely to ingest more blood and lay more eggs than the small ones [**39**]. In the present study, comparatively larger female body size and higher egg production were recorded for *Ae. aegypti* developed from Diet 1 (Micro-180) and it was 4.52 eggs/female/day over 15 days of egg collection. Whereas egg/female/day was recorded as 2.81 for *Ae. aegypti* reared on for Diet 2 (Super Nova). The percentage of egg hatching was almost similar on both the diet treatments. However, a bigger female size may correlate with higher fecundity and fertility of *Ae. a*egypti as observed in the present study may contribute to the mass production of SIT.

Studies related to the digestion of carbohydrates in mosquitoes have indicated that carbohydrates provide an important source of energy for flight and contribute to the longevity and fecundity of mosquitoes [**40,41**]. The statement supports our findings where a shorter life span (~7days) was recorded for males of *Ae. aegypti* has given access to water only than males that had access to sugar solution. The longevity of males (32-36 days) that had access to sugar solution was almost similar and no significant differences were observed between the diet treatments (d=1,4 F=2.58; P=0.184).

A larval diet for mosquitoes must provide sufficient and balanced amounts of nutrients to successfully support not only larval growth but also the production of adults of high quality. Commercial animal diet products have been historically the major components of mosquito rearing diets because they are inexpensive, more homogeneous than natural food sources, and easy to acquire and manage in large quantities [**42-44**]. Our study showed that the egg/female/day by *Ae. aegypti* reared on the Micro-180 diets was higher compared to our Lab diet (Super Nova). Therefore, the effectiveness of the Micro-180 diet may be attributed to its high nutritional value. Although adult *Ae. aegypti* showed almost similar life span when reared on Diet 1 and Diet 2. Diet 1 (Micro-180) contains

high amounts of proteins, fats and calcium than Diet 2 (super Nova). It is not clear how variations in each nutrient influence allocation to various mosquito functions. In this study, diet treatments did not affect the ability of mosquitoes to escape the flight tubes. The flight ability of males *Ae. aegypti* reared on the Micro-180 diet did not differ from those reared on Diet 2. Mating capacity in terms of copula formation was also not affected by the present diet treatments. These evaluations have important implications in the design and planning of operational SIT activities.

Conclusion

The present findings revealed that both the diet treatments, i.e., Diet 1 (Micro-180) and Diet 2 (Super Nova) could prove valuable for mass rearing of *Ae. aegypti* for use in SIT programs, or other types of vector control programs that require the mass-release of this species. However, the fast development and emergence of adult males before adult females (protandry) is a form of sexual selection for increased male mating opportunities via access to virgin females [58], so the search for diets that induce the expression of this trait is important. Moreover, recently the use of insects to feed insects has received tremendous response in several courtiers and become attractive as an alternative ingredient to reduce the rearing cost of different mosquito spp. In future, such an attempt should be taken to reduce the rearing cost as well as improve quality mosquitoes targeting for SIT application.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	NS	MK	MRK	NK
Research concept and design	--	✓	--	--
Collection and/or assembly of data	✓	--	--	✓
Data analysis and interpretation	✓	✓	--	✓
Writing the article	✓	✓	--	--
Critical revision of the article	✓	✓	✓	--
Final approval of article	✓	✓	✓	✓
Statistical analysis	--	✓	--	--

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