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Exploiting nutrition for sex separation in *Culex quinquefasciatus* (Say) species of mosquitoes

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Abstract
The experiment was conducted to exploit nutrition for sex separation in C*ulex quinquefasciatus* at Nuclear Institute for Food and Agriculture (NIFA), Peshawar during 2015. Seven different diets with various concentrations comprising of carbohydrates and protein sources in individual/mixed form were compared with standard diet of IAEA. Maximum pupae size of 3.00 and 3.51 mm for male, 4.01 and 4.40 mm for female, adult's emergence of 45.15 and 70.10% for male were recorded when IAEA diet was tested at 1 and 2% concentration, respectively. Female adult's emergence of 55.17 and 60.25% was recorded when chickpea was fed to larvae at both the concentrations, respectively. Shorter larval period of 7.06 and 7.00 days was noted in larvae nourished by IAEA diet at both concentrations, respectively. Carbohydrates based diet favored male size while, protein skewed in favor of female. It is concluded that the finding on the sexual dimorphism can be exploited for the sex separation of *Culex* and other species of mosquitoes for the successful application of Sterile Insect Techniques.

Keywords: *Culex quinquefasciatus*, diets, pupae size, larval period, sex separation, sterile insect techniques

1. Introduction
Mosquitoes are dipterous insects that are classified in 39 genera with more than 3000 species in the world [1]. *Culex*, *Aedes* and *Anopheles* are three genera that are key vectors of different diseases like dengue hemorrhagic fever, yellow fever, malaria, filariasis, Japanese encephalitis, chikungunya and West Nile virus [2]. These vectors breed in natural as well as artificial containers for instance pools, septic tanks, gutters, tree holes, coconut shells, leaf axils and so on [3].

Mosquitoes are the mainly disreputable ones amongst the most important vectors of human diseases. Atmospheric variation, urbanization in addition to globalization have picked up the pace spread resulting increased number of eruptions of novel mosquito borne diseases, particularly dengue [4]. Most of mosquito transmit diseases exceed to more than 700 million people once a year [5]. Dengue fever and dengue hemorrhagic fever both are vector borne ailments of public health significance in humid, sub-humid, and clement regions. Millions of people get affected by these diseases every year [6]. The annual incidence of about 50 million cases with 500,000 severe cases is estimated as the probable spreading of this arboviral disease worldwide [2].

In consideration of the problems linked with predictable mosquitoes management, for example insect resistance and possible negative side-effects on human health associated with insecticide use, enormous efforts are necessary to build up new or else balancing management techniques for most important mosquito species and sterile insect technique. A number of populations are affected by vector borne diseases every year, so vector control including both anti-larval and anti-adult events comprises an imperative characteristic of any mosquitoes control program. The use of synthetic insecticides is an effective vector control strategy expansively in daily lives which are still at the vanguard of mosquito-controlling efforts. Though, the ecological danger with the intention of these chemicals affects non-target organisms, confrontation of mosquitoes to insecticides have all increased throughout the last five decades [7].

The utilization of radiation in the form of Sterile Insect Technique (SIT) all the way through laboratory reared sterile males is the latent alternative choice for the dengue vector control [8]. This technique has been used widely to control a number of insect pest species [9]. The let go of only males is a requirement for winning as well as efficient SIT program of mosquitoes, therefore a well-organized sex separation system is necessary.

Male mosquitoes are usually smaller than female resultant in smaller pupae and a shorter progress time. Nutritional, behavioral and developmental tools are likely to increase than inherited methods and could be developed as efficient system for eradicating females in the embryo phase. Sex separation is possible at the pupal stage due to size dimorphism between male and female pupae, females are generally larger [10].

Besides having a larger width than larvae, pupae are sexually dimorphous in mass to unreliable extents by means of males being smaller than females. Size separation has then been a useful pupae compilation and sexing method in the laboratory with in small factory settings. Pupae can be separated from larvae regardless of the species but the rigidity for sex separation of pupae is resolute by the built-in difference in the degree of size distinction. The visual severance has been used to select male *C. quniquefasciatus* pupae by hand in its most simple form [11]. Mosquito pupae sexual dimorphism has showed functional sexing technique at the pupae phase. Keeping in view, the mounting tendency of high mortality from mosquito species vectoring pathogens, there is a dreadful need to formulate setting up for its management and subsequent to nutritional methods of sex separation in *C. quinquefasciatus* species of mosquitoes.

2. Materials and methods

The experiment was conducted for one year. An experiment to exploit nutrition for sex separation in *C. quinquefasciatus* species of mosquitoes at Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar during 2015.

2.1 Rearing procedures (Mosquitoes culture)

Stock culture of mosquitoes was established by collecting the larvae from the different breeding habitats. Larval and pupae collections were made with 0.5 liter iron dippers. The larvae were collected in plastic bottle (2L). The collected larvae were brought to the laboratory while established the culture under lab condition. *C. quinquefasciatus* larvae and pupae were collected from different breeding places i.e. irrigation channels, drainage channels and river banks etc. The collection was brought to the Medical Laboratory at Entomology Division of NIFA. The culture was placed in plastic tubs with 01 liter water to establish mosquito's culture which were covered with nylon cloth and small amount of larval diet was added to each tub. The emerged adults were transferred to transparent Plexiglas cages where they were fed with a 10% (W/V) sugar solution by placing cotton soaked in sugar solution on top of cage where mesh was installed and were also fed with blood artificially through parafilm membrane stretched over aluminum plate. The temperature of lab was maintained 26±2ºC and Relative humidity at 65±5%. The culture was established by following the standard mosquitoes rearing procedures of [12]. Identification of species was made with the help of available taxonomic keys [13, 14]. Owing to the reproductive difference and food requirements, female's adults prefer to consume more proteinaceous food and blood meal especially for embryonic development of new batches of eggs while male survival depend on feeding upon high grade of carbohydrate contents. This hypothesis is being tested at the larval stage of *Culex* spp. whether this differentiation is present at the larval stages or not.

Various larval diets consisting of wheat, rice, maize and chickpea, bean, peanut were grinded and changed to powder form and one standard diet IAEA (consisting of 14% brewer's yeast + 36% bovine liver + 50% tuna meal and as an additive 0.2g of Vitamin mix) was used. One and two percent diet concentration was made by dissolving 1 and 2 gram of powder ingredients in a 100ml of distilled water, respectively. Two concentrations of each diet were tested as 1% and 2% (W/V) solutions. Mix diets consisting of Wheat, rice, maize (Carbohydrate) and chickpea, bean, peanut (Protein) were also used for experiment in different ratios (1:1), (1:2) and (2:1). These diets after preparation was put in gas jar and stored in refrigerator following IAEA guidelines [15]. For this purpose 25 1st instar larvae were put in plastic cup containing 100ml of water (tap water) and replicated four times for each treatment. 0.1ml of diet was provided from each concentration to the rearing larvae. The required amount of each diet was put in each cup having culture of larvae with the help of pipette on daily basis till pupae emergence. After pupae emergence, the larval duration was recorded; pupae size was observed under stereo microscope (Stereo-microscope, Optica, SZMA1, Italy). Each pupae was then transferred to separate plastic cups after size measurement and labeled with their respective treatment and replication. Percent male and female adult emergence was recorded after pupae change into adult form from each cup for every treatment, which was covered with nylon cloth. The following parameters were studied during experimental period.

Size of male pupae (mm)

Size of female pupae (mm)

$$\text{Percent male adult emergence} = \frac{\text{No. of male emerged}}{\text{Total no. of pupae}} \times 100$$

$$\text{Percent female adult emergence} = \frac{\text{No. of female emerged}}{\text{Total no. of pupae}} \times 100$$

Larval period (days)

2.2 Statistical analysis

The experiment was laid down in complete randomized design (CRD). The collected data were subjected to ANOVA and means were separated using least significant difference (LSD) test [16].

3. Results

Experiment on the nutritional methods of sex separation in mosquitoes was conducted at Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar during 2015. Seven larval diets at two concentrations i.e. 1% and 2% were applied against mosquito's population. The results are presented in Tables 1 to 5.

3.1 Pupae size at 1% & 2% diet concentration

Significant differences in pupae size of male mosquitoes were recorded when diets were provided at 1% concentration. Maximum pupae size (3.00mm) of male mosquitoes was recorded when the larvae were fed on IAEA diet followed by wheat and rice with pupae size of 2.93 mm and 2.89 mm, respectively (Table 1). Minimum pupae size (2.40 mm) was recorded when the larvae were nourished with peanut. The pupae size of male mosquitoes (2.73 mm, 2.60 mm and 2.51 mm) was perceived by maize, chickpea and bean, respectively. The pupae size of female mosquitoes was significantly different when pupae were fed on 1% diet concentration. Maximum pupae size (4.01 mm) of female mosquitoes was recorded when the larvae were provided for IAEA diet, followed by wheat (3.53 mm), (Table 1). The subsequent female pupae size (3.42 mm) was recorded when the larvae were fed on a diet solution of rice. Minimum size of female pupae (2.21 mm) was recorded when the larvae were nourished with peanut diet. The size of female mosquito pupae

(3.08 mm, 2.81 mm and 2.60 mm) was perceived by maize, chickpea and bean, respectively.

Table 1 further showed considerable variation in pupae size of male mosquitoes when fed at 2% diet concentration, however pupae fed with wheat and rice were statistically at par with each other in size. Likewise, pupae fed with bean (2.56 mm) and peanut (2.49 mm) were non-significant. The increase in diet solution resulted in larger size of male pupae (3.51 mm) when the larvae were provided IAEA diet, followed by wheat and rice with pupae size of 3.11 mm and 3.02 mm, respectively (Table 1). Smaller size of male mosquito's pupae (2.49 mm and 2.56 mm) was recorded when the larvae were fed with peanut and bean based diets. No significant difference in size of male pupae was recorded (2.78 mm and 2.68 mm) when larvae were nourished with maize and chickpea diets. The size of female pupae was significantly different when fed on 2% diet concentration, however, larvae nourished with rice and maize were non-significant in pupae size (3.83 mm). Maximum pupae size (4.40 mm) of female mosquitoes was noticed when the larvae were provided with IAEA diet. The succeeding female pupae of 3.91 mm,3.88 mm and 3.83 mm were recorded when the larvae were fed on a diet solution of wheat, maize and rice, these being non-significant. Minimum size of female mosquito pupae (3.35 mm) was recorded when the larvae were nourished with peanut diet solution.

Table 1: Effect of different larval diets at 1% and 2% concentration on size of pupae of *Culex* spp. during 2015

Larval diets	Pupa size (mm)			
	Male		Female	
	1%	2%	1%	2%
Wheat	2.93 a	3.11 b	3.53 b	3.91 b
Rice	2.89 ab	3.02 b	3.42 bc	3.83 bc
Maize	2.73 bc	2.78 c	3.08 cd	3.88 bc
Chickpea	2.60 cd	2.68 cd	2.81 de	3.69 cd
Bean	2.51 de	2.56 d	2.60 ef	3.55 de
Peanut	2.40 e	2.49 d	2.21 f	3.35 e
IAEA	3.00 a	3.51 a	4.01 a	4.40 a
LSD (0.05%)	0.18	0.20	0.39	0.21

Means in column followed by similar letters are not significantly different at 0.05% level of probability (LSD test)

3.2 Adult's emergence at 1% and 2% diet concentration

The adult's emergence of mosquitoes varied with different diets of carbohydrate, protein and IAEA fed to larvae at 1% concentration. Maximum male adult's emergence (45.15%) was recorded when larvae were reared with IAEA diet, followed by wheat and rice indicating the adult emergence of 42.17 and 41.08%, respectively (Table 2). Minimum adult's emergence (8.25%) of males was noticed in peanut based diet. The emergence of male adult mosquitoes was as 39.17%, 15.15% and 12.20% by nourishing of maize, chickpea and bean diets. Significant differences were observed in female adult's emergence at 1% concentration, however, the female adult emergence was maximum when larvae were fed with chickpea (55.17%), followed by bean (50.17%) and peanut (40.13%) diets. Minimum female adult's emergence (5.10%) was recorded when nourished with maize diet, followed by adult's emergence (7.10%) when fed with rice based diet, these being non-significant from each other. Wheat and IAEA diets showed similar female's adult emergence (10.00%) and (12.18%), respectively.

Male adult's emergence of mosquitoes significantly varied by feeding larvae with different carbohydrate, protein and IAEA based diet at 2% concentration, (Table 2). Maximum male adult's emergence (70.10%) was recorded when IAEA diet was fed to larvae, followed by wheat with adult's emergence of (67.13%). Minimum and non-significant male adult emergence (15.23% and 16.17%) was recorded in peanut and bean based diets. The emergence of male adult's mosquitoes was recorded 65.13%, 60.15% and 20.13% when larvae were provided rice, maize and chickpea, respectively. Considerable variations were observed in female adult's emergence when the larvae were provided 2% concentration of the tested diets. However, larvae fed with rice, maize, wheat and IAEA were statistically at par (Table 2). Maximum female adult's emergence was recorded by giving protein diet to larvae as chickpea (60.25%), followed by bean (54.15%) and peanut (50.25%) Minimum female adult's emergence (10.05%) was recorded when maize diet was provided to larvae. The larvae fed with wheat (15.00%) and IAEA diet (15.10%) showed non-significant variation in adult emergence. Similarly, adult's emergence of female mosquito was also non-significant when provided with rice (12.00%) and maize (10.05%).

Table 2: Effect of different larval diets at 1% and 2% concentration on adult emergence of *Culex* spp. during 2015

Larval diets	Percent adult emergence			
	Male		Female	
	1%	2%	1%	2%
Wheat	42.17 b	67.13 ab	10.00 d	15.00 d
Rice	41.08 bc	65.13 b	7.12 e	12.00 e
Maize	39.17 c	60.15 c	5.10 e	10.05 e
Chickpea	15.15 d	20.13 d	55.17 a	60.25 a
Bean	12.20 e	16.17 e	50.17 b	54.15 b
Peanut	8.25 f	15.23 e	40.13 c	50.25 c
IAEA	45.15 a	70.10 a	12.18 d	15.10 d
LSD (0.05%)	2.65	3.32	2.41	2.59

Means in column followed by similar letters are not significantly different at 0.05% level of probability (LSD test).

3.3 Pupae size at 1% and 2% mixed diet solution

Significant differences were observed in pupae size of male mosquitoes when larvae were fed in different ratios of carbohydrates and protein at 1% diet concentration. It was found that food ratios of 1:1 and 1:2 were statistically at par with each other (Table 3). However, maximum pupae size of male (2.95 mm) was noted when carbohydrates and protein were mixed in 2:1. While minimum pupae size of male (2.41 mm) was recorded after mixing of food ingredients as carbohydrates and protein in 1:1. The effect of mix larval diets on pupae size of female mosquitoes indicated considerable differences when larvae were fed diets in different ratios of carbohydrates and protein at 1% concentration. Results showed that food mix in 1:1 and 1:2 were statistically parallel with each other (Table 3). However maximum pupae size of female mosquitoes (3.90 mm) was noted when carbohydrates and protein were mixed in 2:1 and minimum pupae size of female mosquitoes (3.33 mm) was recorded after mixing of food ingredients as carbohydrates and protein in 1:1.

Results presented in Table 3 shows the effect of 2% mix diet solution on pupae size. However, food mix in 1:1 and 1:2 were statistically at par with each other. Maximum pupae size of male (3.21 mm) was noted when carbohydrates and protein were mixed in 2:1. Minimum pupae size of male (2.70 mm) was recorded after mixing of food ingredients as carbohydrates and protein in 1:1, followed by pupae size of 2.85 mm when foodstuff of carbohydrates and protein was mixed in 1:2. Maximum pupae size of female (4.13 mm) was recorded when carbohydrates and protein were mixed in 2:1. The minimum pupae size of female mosquitoes (3.54 mm) was recorded when food ingredients as carbohydrates and protein in 1:1 fed

to the larvae followed by pupae size of (3.80 mm) when diet was mixed in 1:2. These were found non-significant from each other.

Table 3: Effect of mix larval diets at 1% and 2% concentration on size of pupae of *Culex* spp. during 2015

Larval diets	Pupa size (mm)			
	Male		Female	
	1%	2%	1%	2%
Wheat, rice, maize (carbohydrates) : Chickpea, bean, peanut (protein) (1:1)	2.41 b	2.70 b	3.33 b	3.54 b
Wheat, rice, maize (carbohydrates) : Chickpea, bean, peanut (protein) (1:2)	2.61 b	2.85 b	3.55 b	3.80 b
Wheat, rice, maize (carbohydrates) : Chickpea, bean, peanut (protein) (2:1)	2.95 a	3.21 a	3.90 a	4.13 a
LSD (0.05%)	0.21	0.30	0.22	0.30

Means in column followed by similar letters are not significantly different at 0.05% level of probability (LSD test).

3.4 Adult's emergence at 1% and 2% mix diet solution

The effect of mix larval diets on the emergence of male adult's mosquitoes showed significant variations when larvae were fed diets in different ratios of carbohydrates and protein at 1% diet concentration (Table 4). Maximum male adult's emergence (52.67%) was noted when carbohydrates and protein were mixed in 2:1. Minimum male adult's emergence (20.00%) was recorded after mixing of food ingredients as carbohydrates and protein in 1:2 at 1% diet feeding. The adult emergence of male mosquitoes was 31.67% when carbohydrates and protein were mixed in 1:1. Female adult's emergence at 2% diet concentration showed that the maximum female adult emergence was recorded (42.33%) when carbohydrates and protein were mixed in 1:2. Minimum female adult's emergence (15.47%) was recorded after mixing of food ingredients as carbohydrates and protein in 2:1 followed by diet mixed in 1:1 (24.33%).

Results regarding the adult's male emergence at 2% mix diet solution are presented in Table 4. It was found that maximum male adults emergence (57.57%) was recorded when carbohydrates and protein were mixed in 2:1 and minimum male adult's emergence (22.13%) was recorded after mixing of food ingredients as carbohydrates and protein in 1:2 followed by adult emergence (32.63%), when diet was mixed at 1:1. Maximum female adults emergence (45.23%) was recorded when carbohydrates and protein were mixed in 1:2, while minimum female adults emergence (17.60%) was recorded when food items as carbohydrates and protein were mixed in 2:1, followed by the diet having carbohydrates and protein mixed in 1:1 (25.80%)

Table 4: Effect of mix larval diets at 1% and 2% concentrations on adult emergence of *Culex* spp. during 2015

Larval diets	Percent adult emergence			
	Male		Female	
	1%	2%	1%	2%
Wheat, rice, maize (carbohydrates) : Chickpea, bean, peanut (protein) (1:1)	31.67 b	32.63 b	24.23 b	25.80 b
Wheat, rice, maize (carbohydrates) : Chickpea, bean, peanut (protein) (1:2)	20.00 c	22.13 c	42.33 a	45.23 a
Wheat, rice, maize (carbohydrates) : Chickpea, bean, peanut (protein) (2:1)	52.67 a	57.57 a	15.47 c	17.60 a
LSD (0.05%)	4.13	6.33	2.29	2.56

Means in column followed by similar letters are not significantly different at 0.05% level of probability (LSD test).

3.5 Larval period

Results regarding larval duration of mosquitoes tested against various diets indicated significant variations. Longer larval period (11.69 days) was recorded for peanut followed by bean (10.13 days) diet fed to larvae. Shorter larval duration was recorded for IAEA diet (7.06 days), followed by wheat (7.37 days) tested at 1% diet concentration. However, larval duration recorded when larvae fed with rice, maize and chickpea based diets were statistically at par with each other (Table 5). Significant differences were observed for larval period of mosquitoes fed on different diets at 2% concentration. Longer larval period (9.75 days) was recorded for peanut diet. Shorter larval duration was recorded for IAEA diet (7.00 days, followed by wheat (7.12 days) and rice (7.25 days), these being non-significant from each other. However, wheat, rice and IAEA diet were statistically at par with each other (Table 5).

Table 5: Effect of different larval diets concentrations on larval duration of mosquito *Culex* spp. during 2015

Larval diets	Larval period (days)	
	1%	2%
Wheat	7.37 d	7.12 c
Rice	8.50 c	7.25 c
Maize	9.00 c	8.12 b
Chickpea	9.12 c	8.50 b
Bean	10.13 b	8.88 b
Peanut	11.69 a	9.75 a
IAEA	7.06 d	7.00 c
LSD (0.05%)	0.74	0.86

Means in column followed by similar letters are not significantly different at 0.05% level of probability (LSD test).

4. Discussion

In the present study, three larval diets of carbohydrates, three larval diets of protein and one standard IAEA diet were tested in two concentrations individually while carbohydrates and protein based diets were also used in combination for rearing *Culex quinquefasciatus* mosquitoes. These diets were tested in individual and mixed ratios in different concentration for pupae size, adult's emergence and larval duration of male and female rearing for sex separation of mosquitoes. Pupae size of males at 1% diet concentration significantly affected by larval diets. Providing carbohydrate diets enlarged the pupae size of male compared to protein diets. Maximum pupae size was noted when the larvae were fed on IAEA diet, subsequent to wheat and rice (carbohydrates). Minimum size of males at pupae stage was recorded when the larvae were nourished with peanut (protein) diet solution. However, it appeared that food diets of maize (carbohydrates), chickpea and bean (protein) showed no or least difference in size of male mosquitoes. The reason may be due to the distinction in the nutritive value of various diets [17]. The pupae size of female mosquitoes was significantly different when pupae were fed on 1% diet concentration. Maximum pupae size of female mosquitoes was noticed when the larvae were provided for IAEA diet. The subsequent size of female pupae was recorded when the larvae were fed on a diet solution of rice (carbohydrates). Minimum size of female pupae was recorded when the larvae were nourished with peanut (protein). The least difference in size of female pupae was observed by maize (carbohydrates), chickpea and bean (protein). It became visible that food diets containing carbohydrates enlarged pupae size of male and female mosquitoes at 1% concentration. The role of natural

ingredients in diet worked for the growth and size of mosquitoes, *An. arabiensis* [12].

At diet concentration of 2%, considerable variation was observed in pupae size of male pupae. It was observed that amplified diet solution of IAEA, wheat and rice increased size of male mosquitoes. Diet reserves were significantly higher in males reared with carbohydrates compared to those in females reared with protein. Pupae fed with protein (bean and peanut) were of the same manner in size resulting smaller size of male pupae. The vitamin level supplements a vital role in the normal growth and survival of *Culex pipiens* [18]. The size of female pupae was significantly different when fed on 2% diet concentration. Maximum pupae size of female mosquitoes was noticed when the larvae were provided for IAEA diet. Diet solution of peanut (protein) showed smaller size of female pupae. Female reared with IAEA, wheat and rice accumulated more carbohydrates than those reared with bean, peanut (protein) diets resulted large pupae size. This may be due to sufficient amount of vitamins and carbohydrate cereals indicating large size of pupae. Size differentiation for sex separation can be obtained by mixed diets of tuna meal, bovine liver powder and brewer's yeast [19].

Emergence of adult mosquitoes varied with different diet of IAEA fed to larvae at 1% concentration. Maximum male adults emergence was noted when food diet of IAEA was applied against the insects. The difference in emergence may be due to genetic variance in mosquito's species. Diet, density, and temperature influenced the difference in development rate and emergence [20]. Significant differences were observed in female adult's emergence as a result of food provided to larvae. The female adult's emergence was greater by giving protein diet to larvae as chickpea and bean. The larvae fed with wheat and IAEA diets showed lower emergence. The adult's emergence of female mosquitoes was slight by peanut (protein) diet at 1% concentration. The difference in appearance may be the fact that cereal crops have more protein diets resulting better adult's emergence of female mosquitoes. Females reared on bovine liver powder mixed with various food diets achieved best during larval emergence [21].

Significant differences were observed for male adult's emergence by feeding larvae with diet of IAEA, carbohydrate and protein at 2% concentration. Maximum male adult's emergence was noted when IAEA diet was fed to larvae. Male adult's emergence was best by nourishing of maize (carbohydrates) and chickpea (protein) diets at 2% concentration. It may be suggested that IAEA diets have more vitamins resembling to male adults showing faster emergence. Infact, more energy is stored as carbohydrates during larval stage [22]. Considerable variations were observed in female adult's appearance. Maximum female adult's emergence was recorded by giving protein diet to larvae as chickpea. The larvae fed with wheat and IAEA diets showed low emergence. Best emergence of female mosquitoes was recorded when larvae were fed with peanut (protein) diet at 2% concentration. Females reared on bovine liver powder mixed with various food diets achieved best during larval emergence [21].

Larval duration of mosquitoes was significant when larva were fed with various food diets of carbohydrates and protein at 1% concentration. As compared to other food items, shorter larval duration was recorded for IAEA diet. This could be made clear by the shorter larval development time, meaning that males from this action (IAEA diet) did not build up as many assets during larval stages. Longer larval period was recorded for peanut and bean (protein) diets fed to larvae. Food diets of chickpea, maize and rice showed slight larval duration when larvae were fed at 1% concentration. Providing IAEA diet a shorter progress time would produce a short rearing schedule for obtaining the let go of male mosquitoes. Significant differences were observed for larval period of mosquitoes fed on different diets carbohydrates and protein at 2% concentration. Shorter larval duration was recorded for IAEA diet, wheat and rice at 2% concentration. Longer larval period for peanut diet; however, food diets of bean, chickpea and maize showed non-significant larval period at 2% concentration. Highest larval with pupae development and maximum survival rates were observed by feeding diets of bean, corn, wheat, chickpea and rice [23].

Mixed larval diets showed significant variations on pupae size of male and female at 2:1 ratios. It is important to note that segregation in nourishment as higher rate of carbohydrates may cause difference in size resultant higher pupae size in males. Differences in intrinsic size, performance and progress rate by females and males which were often accessible and beneficial for sexing [24]. Maximum pupae size of female mosquitoes was noted when carbohydrates and protein were mixed in 2:1 at 1% diet solution. The results showed that doubled food energy of carbohydrates fed to males and females resulted higher size of pupae in mosquitoes. The role of natural ingredients in diet worked for the growth and size of mosquitoes, *An. arabiensis* [12]. Mix larval diets on pupae size of male and female mosquitoes showed significant variations when they were fed at 2% diet concentration. Maximum size of male pupae was noted when carbohydrates and protein were mixed in 2:1. This showed that doubled food energy in form of carbohydrates may augment the size of pupae at higher concentration. Differences in intrinsic size, performance and progress rate by females and males were found which were often accessible and beneficial for sexing [24]. Maximum pupae size of female mosquitoes was noted when carbohydrates and protein were mixed in 2:1 at 2% diet concentration. The mixing of food diets with high rates of carbohydrates enlarged the pupae size of female mosquitoes at binary concentration. Size differentiation for sex separation can be obtained by mixed diets of tuna meal, bovine liver powder and brewer's yeast [19].

Mixed larval diets showed significant effect on the emergence of adult mosquitoes at 1% diet concentration. Maximum male adults emergence was noted when carbohydrates and protein were mixed in 2:1. The difference in emergence may be due to genetic variance in mosquito's species as well as nutritional differentiation in diets. Many mosquitoes emerge with carbohydrates diet accumulated in larval stage [25]. Maximum female adults emergence was noted when carbohydrates and protein were mixed in 1:2 at 1% diet concentration. It was seen that the addition of carbohydrates increased the materialization of female mosquitoes. The highest adult's emergence was observed for larvae fed with mixture of locally available diets [12].

Mixed food diets in diverse ratios tested against adult's emergence of mosquitoes showed significant effect at 2% concentration. Maximum male adults emergence was noted when carbohydrates and protein were mixed in 2:1. It is obvious from the data that IAEA diets were rich in nutritious status showing faster emergence of male mosquitoes. Maximum mosquitoes emerge with diet rich in carbohydrates in larval stage [26]. Female adult's emergence was higher when carbohydrates and protein were mixed in 1:2 at 2% diet solution. Mixed diets of carbohydrates and protein in 1:2 provided sufficient energy for improved survival rate at doubled concentration indicating higher adult's emergence. The combination of two components containing bovine liver

powder and tuna meal (protein) showed the most admirable results in terms of emergence [27].

5. Conclusion

On the basis of findings obtained in this study, it is concluded that food diets provided by IAEA along with wheat showed better performance against size of both the sexes tested at each two concentrations. IAEA diets showed better adults emergence in male, while chickpea diet exhibited higher adults emergence in female at both concentrations. Larval period was shorter in IAEA diets and longer in peanut diets fed to larvae. Mixed diets of carbohydrates and protein in 2:1 amplified the size of both male and female mosquitoes. The adult's emergence of male was higher in diets at 2:1 and female in 1:2 of carbohydrate and protein at both the concentrations.

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7. References

1. Polson KA, Rawlins SC, Brogdon WG, Chadee DD. Characterization of insecticide resistance in Trinidadian strains of Aedes aegypti mosquitoes. Acta Tropica. 2011; 117(1):31-38.
2. World Health Organization (WHO). Dengue and dengue hemorrhagic fever. World Health Organization. Media centre Fact Sheet. 2008.
3. Aigbodion FI, Anyiwe MA. Mosquitoes and environments: some financial costs of malaria in Nigeria. Nigerian Journal of Entomology. 2005; 22:93-107.
4. Kyle JH, Harris E. Global spread and persistence of dengue. Annual Review of Microbiology. 2008; 62:71-92.
5. Aregawi M, Williams R, Dye C, Cibulskis R, Otten M. World Malaria Report, 2008. World health organization, Geneva. 2008, 118.
6. Gibbons V, Vaughn G. Dengue: An Escalating Problem. British Medical Journal. 2002; 324:1563-1566.
7. Amer I, Mehlhorn F. Repellency effect of forty one important oil against Aedes, Anopheles and Culex mosquitoes. Parasitology. Residence. 2006; 99(4):478-490.
8. Catteruccia F, Crisanti A, Wimmer EA. Transgenic technologies to induce sterility. Malaria Journal. 2009; 8(2):117-118.
9. Dyck VA, Hendrichs J, Robinson A. Sterile insect technique: principles and practice in area wide integrated pest management, 2005, 17-87.
10. Papathanos PA, Bossin HC, Benedict MQ, Catteruccia F, Malcolm C, Alphey L *et al*. Sex separation strategies: past experience and new approaches. Malaria Journal. 2009; 8:S5.
11. Krishnamurthy BS, Ray SN, Joshi GC. Note on preliminary field study of the use of irradiated males for reduction of Culex fatigans Wied population. Indian Journal of Malaria. 1962; 16:365-373.
12. Khan M, Vreysen J. Comparing efficacy of mixed larval diets on the developmental attributes of Anopheles arabiensis Patton. Pakistan Journal of Zoology. 2011; 43(6):1111-1115.
13. Hopkins GHE. Mosquitoes of the Ethiopian region 1, - Larval bionomics of mosquitoes and taxonomy of culicine larvae, 2nd ed. British Museum (Natural History), London, 1952, 355.
14. Highton RB. Taxonomic keys for the identification of the afrotropical mosquitoes. Unpublished, 1983, 1-85.
15. Puggioli A, Balestrino F, Damiens D, Lees RS, Soliban SM, Madakacherry Q *et al*. Efficiency of three diets for larval development in mass rearing. Aedes albopictus (Diptera: Culicidae). Journal of Medical Entomology. 2013; 50(4):819-825.
16. Steel RGD, Torrie JH. Principles and procedures of statistics: A biometrical approach. 2nd Edition, McGraw Hill Book Co., New York, 1980.
17. Iqbal A, Khalil IA, Ateeq N, Khan MS. Nutritional quality of imported food legumes. Food Chemistry. 2006; 97:331-335.
18. Dadd RH, Kleinjan JE, Asman SM. Eicosapentaenoic acid in mosquito tissues: differences between wild and laboratory reared adults. Environmental Entomology. 1988; 17:172-180.
19. Balestrino F, Puggioli A, Gilles JRL, Bellini R. Validation of a new larval rearing unit for Aedes albopictus (Diptera: Culicidae): Mass Rearing. Journal of Pone. 2014; 9(3):e91914.
20. Couret J, Dotson E, Benedict M. Temperature, larval diet and density effects on development rate and survival of Aedes aegypti. Journal Pone. 2014; 9(2):467-468.
21. Yahouedo GA, Djogbenou L, Saizonou J, Assogba B, Simard F. Effect of three larval diets on larval development and male sexual performance of Anopheles gambiae. Acta. Tropica. 2014; 132:96-101.
22. Nisbet R, Muller E, Lika K, Kooijman S. From molecules to ecosystems through dynamic energy budget models. Journal of Animal Ecology. 2000; 69:913-1839.
23. Khan I, Farid A, Zeb A. Development of inexpensive and globally available larval diet for rearing Anopheles stephensi (Diptera: Culicidae) mosquitoes. Parasites and Vector. 2013; 6(9):33-35.
24. Gilles J, Marc FS, Scolari F, Marec M, Capurro M, Franz G *et al*. Towards mosquito sterile insect programs: Exploring genetic, molecular, mechanical and behavioral methods of sex separation in mosquitoes. Acta Tropica. 2014; 132:S178-S187. http://dx.doi.org/10.1016/j.actatropica.2013.08.015
25. Nayar JK. The biology of Culex nigripalpus Theobald (Diptera: Culicidae), Part 2. Adult characteristic at emergence and adult survival without nourishment. Journal of Medical Entomology. 1968; 5:203-210.
26. Foster WA. Mosquito sugar feeding and reproductive energetics. Annual Review of Entomology. 1995; 40:443-474.
27. Damiens D, Benedict MQ, Wille M, Gilles JRL. An inexpensive and effective larval diet for Anopheles arabiensis: eat like hose, bird or fish. Journal of Medical Entomology. 2012; 49(5):1001-1011.