# SURVIVAL RATE AND DEVELOPMENT OF LARVAE *Elaeidobius kamerunicus* (COLEOPTERA: CURCULIONIDAE) ON ARTIFICIAL DIETS

**Sarah Najihah Mohd Zahari, Rosha Rusli, Mohamad Haris Hussain & Johari Jalinas***
Centre for Ecosystem Management and Natural Resources,
Faculty of Science and Technology, Universiti Kebangsaan Malaysia,
43600 Bangi, Selangor, Malaysia.
*Corresponding email: *johari_j@ukm.edu.my*

## ABSTRAK

Kumbang *Elaeidobius kamerunicus* Faust (Coleoptera: Curculionidae) telah diperkenalkan dalam industri kelapa sawit di Malaysia untuk meningkatkan hasil pokok kelapa sawit. Walau bagaimanapun, terdapat pengurangan pendebungaan terhadap kelapa sawit oleh *E. kamerunicus*. Oleh itu, tujuan kajian ini adalah untuk menghasilkan diet alternatif untuk *E. kamerunicus* bagi mengekalkan larva *E. kamerunicus* di bawah keadaan makmal dan membandingkan perkembangan larva *E. kamerunicus* dalam diet alternatif yang berlainan. Empat jenis diet telah disediakan di mana diet a dan diet b menggunakan bahan seperti ragi, gandum, tepung jagung, asid askorbik, asid benzoik, agar bakteriologi, air suling, metil-4-hidroksibenzoat dan kloramfenikol. Diet c dan d diformulasi dengan ragi, tepung gandum, tepung jagung, madu, serbuk susu dan gliserol. Diet tambahan menggunakan serbuk bunga jantan sawit digunakan untuk diet b dan d. Kadar pupasi dan kemandirian *E. kamerunicus* antara empat diet di bawah keadaan makmal telah direkodkan. Hasilnya menunjukkan bahawa diet d menghasilkan kadar pupasi dan kemandirian tertinggi berbanding dengan diet lain dengan 56% daripada kedua-dua pupasi dan serangga dewasa terhasil daripada 25 larva. Kadar lengkungan kemandirian (y=mx+c) larva *E. kamerunicus* menunjukkan cerun paling rendah dengan nilai m bersamaan dengan -1.2242 yang menunjukkan kadar kemandirian larva tertinggi.

**Kata kunci:** Diet tiruan; *Elaeidobius kamerunicus;* kadar hidup; pupasi; dewasa

## ABSTRACT

The weevil *Elaeidobius kamerunicus* Faust (Coleoptera: Curculionidae) was introduced within the oil palm industry in Malaysia to improve the yield of the oil palm production. However, there were reduction of pollination towards the oil palm plantation by the *E. kamerunicus*. Therefore, the purpose of this study was to develop an artificial diet for *E. kamerunicus* to maintain larvae and compare the development under laboratory conditions in different artificial diets. Four types of artificial diets have been prepared where diet a and diet b used ingredients such as yeast, wheat, corn flour, ascorbic acid, benzoic acid, for bacteriology, distilled water, methyl-4-hydroxibenzoate and chloramphenicol. Diet c and diet d were formulated by yeast, wheat flour, corn flour, honey, milk powder and glycerol. An additional diet which contained oil palm male flowers were introduced in diet b and d. The pupation and survival rate of *E. kamerunicus* between the four diets under laboratory conditions were recorded. The results showed that diet d produced the highest pupation and emerged adults compared to other diets with 56% of both pupation and emerged adults out of total 25 larvae. The survival rate curved (y=mx+c) of larvae *E. kamerunicus* showed the least slope with m value equal to -1.2242 which showed highest survival rate of the larvae.

**Keywords:** Artificial diet; *Elaeidobius kamerunicus;* survival rate; pupation; adult

## INTRODUCTION

Oil palm *Elaeis guineensis* Jacq. (Arecaceae) is one of the important crops for Malaysian economy. It is essentially entomophilous (Syed 1979) and also one of the most extensively cultivated plantation crops in many parts of the world. Pollination by specific insect species is vital for many crops in nature (Sambathkumar & Ranjith 2011). Malaysia reached an area of 5.23 million hectares of oil palm, producing 0.43 million tons in 2013 (MPOC 2014). Previously, wind or insects transferred pollen for the tree to bear male and female flowers on separate inflorescences (Teo 2015). In the beginning, Malaysia's oil palm plantations relied mainly in wind pollination and manually transferred by human but there are also natural Malaysian oil palm insect pollinators such as *Thrips hawaiiensis* (Thysanoptera) and *Pyroderces* sp. (Lepidoptera: Cosmopterygidae). However, they were found to be inefficient (Wahid & Kamaruddin 1997) to pollinate the oil palm plantation. Therefore, to improve the yield, the weevil *E. kamerunensis* have been introduced within the oil palm industry and it is proved to be effectively pollinator (Ponnamma et al. 2006).

According to Syed (1979), pollination of oil palm (*Elaeis guineensis*) is essentially in West Africa and South America respectively. There are 20 species of insects that belong to *Elaeidobius* which are subservient to the flower of the oil palm (Mariau et al. 1991). All the 20 species of the insects do not have the same efficient pollination processes. Four species from the 20 *Elaeidobius*; *E. kamerunicus, E. plagiatus, E. subvittatus* and *E. singularis* have been the largest pollinators of oil palm plantation (Mariau et al. 1991). However, *E. kamerunicus* and *E. plagiatus* are the most active species for pollination (Mariau et al. 1991). The introduction of *E. kamerunicus* address the poor natural pollination problem in Malaysia which is found to be the main pollinator for the oil palm (Syed 1979) and in 1981 it increased pollination and fruit production from 20% to 30% (Syed 1982). *Elaeidobius kamerunicus* is entirely dependent on the male inflorescence of the oil palm to survive because the adult weevils consume oil palm pollen and lay eggs in anthesizing inflorescence.

Recently, there were also declinations of the pollinators such as *E. kamerunicus* in the oil palm plantation which is caused by the excessive use of pesticides such as Cypermethrin and Chlorantraniliprole to control the oil palm insect pest. The pesticides are harmful to *E. kamerunicus* (Yusdayati & Hamid 2015). Subsequently, it brought a negative impact on the pollination activities of *E. kamerunicus.*

Due to economic importance of *E. kamerunicus*, the scarcity of information on its biology and the urgent need to accelerate basic and applied research by human, it is essential to develop an artificial diet for immature stages of the *E. kamerunicus* as alternatives to natural diet. Artificial diets are foods synthesized from one or more ingredients that may be completely defined chemically, partially defined or not defined chemically (Cohen 2004). Artificial diets are used for the domestication, colonization, mass production and maintenance of many of animal species including insects which are important for human welfare. For instance, different types of artificial diets are used to rear and maintain different organism such as fishes (Ruohonen et al. 2003), crustaceans (Catacutan 2002), mollusks (Garcia et al. 2011), echinoderms (Sartori and Gaion 2016), poultry (Basurco et al. 2015) and insects (Cohen 2004). Thus, the aim of this study is to test several formulation of artificial diets for maintaining larvae *E. kamerunicus* survive under laboratory conditions.

## MATERIALS AND METHODS

### Study Site

Study area and field work has been done at selected palm oil plantation to collect the *E. kamerunicus* before conducting experiments. The site chosen was located at Kampung Bukit Changgang Banting, Selangor Darul Ehsan (GPS: 2.837301, 101.610587). The spikelets were also being collected from the oil palm plantation and it had been used to develop new artificial diet of *E. kamerunicus.* The collected spikelets were those already at anthesis and without fungal infection.

### Field Work

Male inflorescences (spikelets) were randomly selected before flowering (pre-anthesis) and were covered with a white cloth to avoid the adult *E. kamerunicus* mating at the male spikelets. All the preparations covered spikelets were left for one week for the spikelets to be fully anthesis before being collected to the laboratory for the next step. The technique used in sampling of the weevil of *E. kamerunicus* was done by using the weevil-covered spikelets of the anthesising male inflorescence. The adult weevils *E. kamerunicus* were collected at the anthesis spikelet in the field.

### Laboratory Work

#### *Insect mating*

Preparation of *E. kamerunicus* larvae samples were obtained by mating between male and female in plastic containers (16.5 cm x 11.5 cm). Five pairs of *E. kamerunicus* were used and five spikelets were placed within each container for feeding and ovi-position process. The insects were maintained in the laboratory under room temperature (25 ± 0.5 ºC). After five days, the spikelets were dissected to get the *E. kamerunicus* larvae for survival and development study in different artificial diets.

#### *Preparation of artificial diets*

The development of the *E. kamerunicus* larvae was studied on different prepared artificial diets of which constituents are given in Table 3.1. Four artificial diets have been formulated to be fed to the larvae of *E. kamerunicus* for the larvae development observation. Diet a and b of the developed artificial diets were mainly composed of brewer’s yeast (Sigma), wheat germ (Oxoid – LP0011, 500g), cornmeal (Sigma, 500g), ascorbic acid (Riendemenn Chmidt, 100g), benzoic acid (Riendemenn Chmidt, 500g), bacteriological agar, distilled water, methyl-4-hydroxybenzoate (Nacalai Tesque, 500g), chloramphenicol (Crystalline, 50g) and only spikelets was added for diet b. However, other artificial diets used the same ingredient but without spikelet. These two artificial diets were prepared with 800 ml of distilled water to the main ingredients to dissolve the 20 g of agar. While diet c and d were composed of honey, wheat flour, milk powder, corn flour, yeast, glycerol and water. Diet d contained the fine grounded of spikelet.

All the artificial diet have their own nutritional value for an insect including the following ingredients; agar is to maintain the humidity of the diets in laboratory, brewer's yeast rich source of minerals while the wheat germ is a concentrated source of several essential nutrients including Vitamin E and it is a good source of fiber. Other ingredient such as corn meal is rich source dietary fiber, a beneficial carbohydrate, iron and phosphorus. Besides that, component methyl-4-hydroxybenzoate that was added in the diet acts as antimicrobial agent while the benzoic acid used as preservative in foods. Ascorbic acid which also known as vitamin C is very important for the growth and repair of tissues while chloramphenicol acts as an antibiotic, sometimes known as a broad spectrum antibiotic as it is effective against a wide variety of bacteria. In artificial diet b and diet d that was added with spikelet, it was used to increase the nutritive value of the diet. The nutritional value of the ingredients for diet c and diet d including the following value; honey contains amino acids, minerals, and vitamin B6, wheat flour mainly contain of carbohydrate, fiber and protein, yeast powder rich of minerals, corn flour contain of carbohydrate, fat and protein while the milk powder contain vitamin, minerals, protein and carbohydrate. Glycerin acts as a solvent in the diet's ingredients and it thickening the diet texture. All the prepared artificial diets were transferred into 1.8 ml of tube for study of EK larva.

Table 1. The different constituent of the suggested developed artificial diet used in larvae development of *Elaeidobius kamerunicus.*

| | Diet a | | Diet b | | Diet c | | Diet d | |
|---|---|---|---|---|---|---|---|---|
| Ingredients | Ingredients | Weight (g) | Ingredients | Weight (g) | Ingredients | Weight (g) | Ingredients | Weight (g) |
| Agar | Bacteriological Agar | 20 g | Bacteriological Agar | 20 g | | | | |
| Water | Distilled Water | 800 g | Distilled Water | 800 g | Distilled Water | 94 g | Distilled Water | 94 g |
| Yeast | Brewer's yeast | 50 g | Brewer's yeast | 50 g | Yeast | 7 g | Yeast | 7 g |
| Fibre | Wheat germ | 50 g | Wheat germ | 50 g | Wheat flour | 192 g | Wheat flour | 192 g |
| Carbohydrate | Corn meal | 50 g | Corn meal | 50 g | Corn flour | 80 g | Corn flour | 80 g |
| Sugar | | | | | Honey | 263 g | Honey | 263 g |
| Preservatives | Benzoic acid | 1.8 g | Benzoic acid | 1.8 g | | | | |
| Vitamin C | Ascorbic acid | 4.5 g | Ascorbic acid | 4.5 g | | | | |
| Vitamin | | | | | Milk powder | 48 g | Milk powder | 48 g |
| Antibiotics | Methyl-4-hydroxybenzoate | 1.8 g | Methyl-4-hydroxybenzoate | 1.8 g | | | | 6 g |
| Antibiotics | Chloramphenicol | 0.5 g | Chloramphenicol | 0.5 g | | | | |
| Solvent | | | | | Glycerol | 208 g | Glycerol | 208 g |
| Host plant | | | Spikelet[a] | 100 g | | | Spikelet[a] | |
| | High-cost diet | | High-cost diet | | Low-cost diet | | Low-cost diet | |

Note: [a] Spikelet was blended until finely ground

***Survival and development of E. kamerunicus larvae***

The 3rd instar larvae of *E. kamerunicus* were used for survival and development of *E. kamerunicus* larvae. Individual larvae were transferred into four groups in separate small tubes(1.8 ml artificial diets) (Fig. 1). The insects were maintained on the same food until the last stage of *E. kamerunicus*'s life cycle. Daily observation was made on larvae development between all four diets. The larvae were observed until they pupated. The pupae formed were then observed until it emerged into adults. The observation of the different development stages was recorded and analyzed on different diets. The data collected were subjected to a comparative statistical analysis using Kaplan-Meier test on the survival rate.

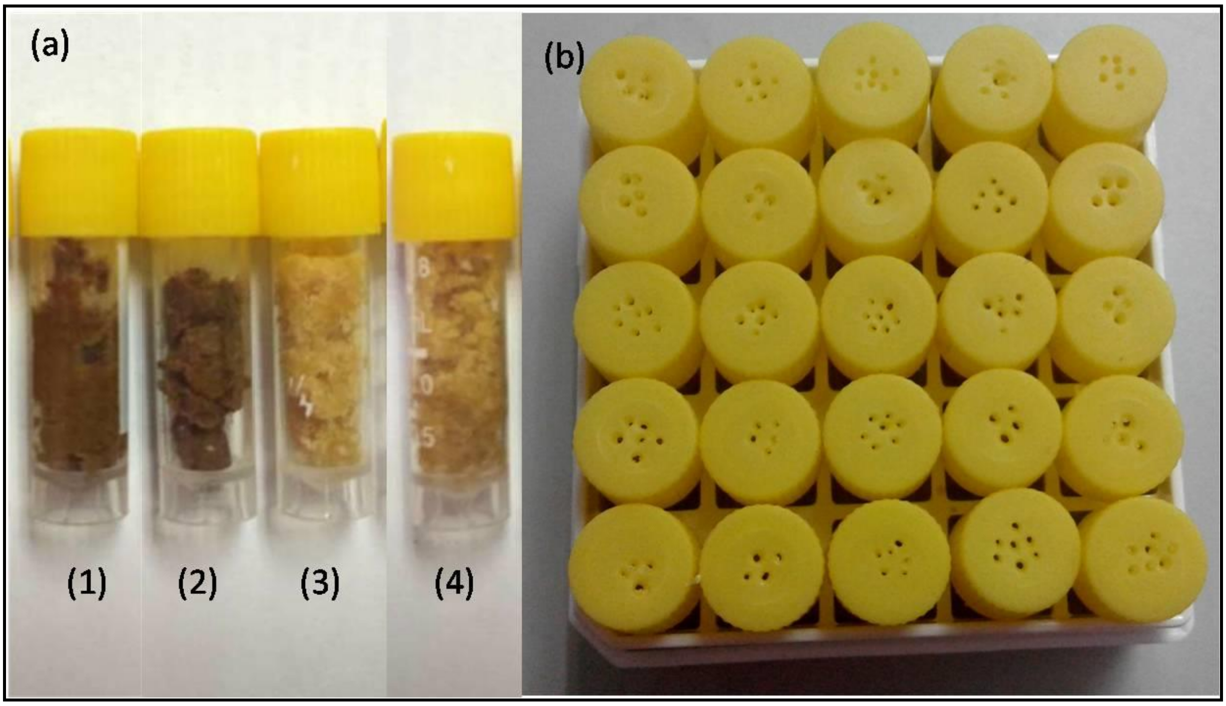


Figure 1. Four artificial diets for feeding of larvae *E. kamerunicus* in laboratory condition.
Note: (a) Different artificial diet: (1) artificial diet a (2) artificial diet b (3) artificial diet c (4) artificial diet d. (b) Example of artificial diet being put in 1.8 ml of small tube.

**Statistical Analysis**

Response variables included larval duration, survival and pupae duration and percentages of emergence of adults. The experimental design is completely random with n= 25. A 1.8 ml tube was filled with developed artificial diet was assigned for each treatment. 25 3rd instar larvae (L3) of *E. kamerunicus* were introduced to each tube, which were kept at room temperature. Kaplan-Meier analysis was used to evaluate differences in each survived larvae between *E. kamerunicus* where there were significant differences tests it employed to describe which means were most alike or different and to test the equality of means for each individual of variables. Linear regression test was done to determine the relationship between survival *E. kamerunicus* with the time (days). All statistical analyses were conducted with Microsoft excel and Kaplan Meier analysis by IBM SPSS statistics 21 software.

## RESULTS

**Development Curved of Larvae of *Elaeidobius Kamerunicus* Treated on Artificial Diet**

This study is about the use of prepared artificial diets for development of larvae of *E. kamerunicus* in laboratory. The study was compared the different developmental stages of larvae in different artificial diets. All the ingredients of the prepared artificial diets were shown in Table 2 which was categorized in four diets. Diet 1 and Diet 2 were modified diet from the study of El-Shafie et al. (2011) while Diet 3 and Diet 4 were modified from the study of Metwally et al. (2012).

A total of 25 larvae of *E. kamerunicus* was used for each artificial diet. Third (3$^{rd}$) larval instar which would take around 5 to 8 days before pupation was used. The percentage (%) number of pupation and emerging adult were shown in Table 2. Figure 2 shows the percentage of pupation of larvae *E. kamerunicus* in four different diets. The percentage number of pupation at diet a, diet b, diet c, diet d and control were 20%, 40%, 32% and 56% respectively. The highest percentage number of pupation observed at diet d, followed by diet b and diet c. The least percentage number of pupation occurred at diet a.

Table 2. Percentage (%) number of pupation and emerging adult of *E. kamerunicus* in different treatments.

| Treatments | Pupation (%) | Emerging adults (%) |
|---|---|---|
| Diet a | 20% | 16% |
| Diet b | 40% | 24% |
| Diet c | 32% | 32% |
| Diet d | 56% | 56% |

Note: No. of inoculated larvae; n = 25.

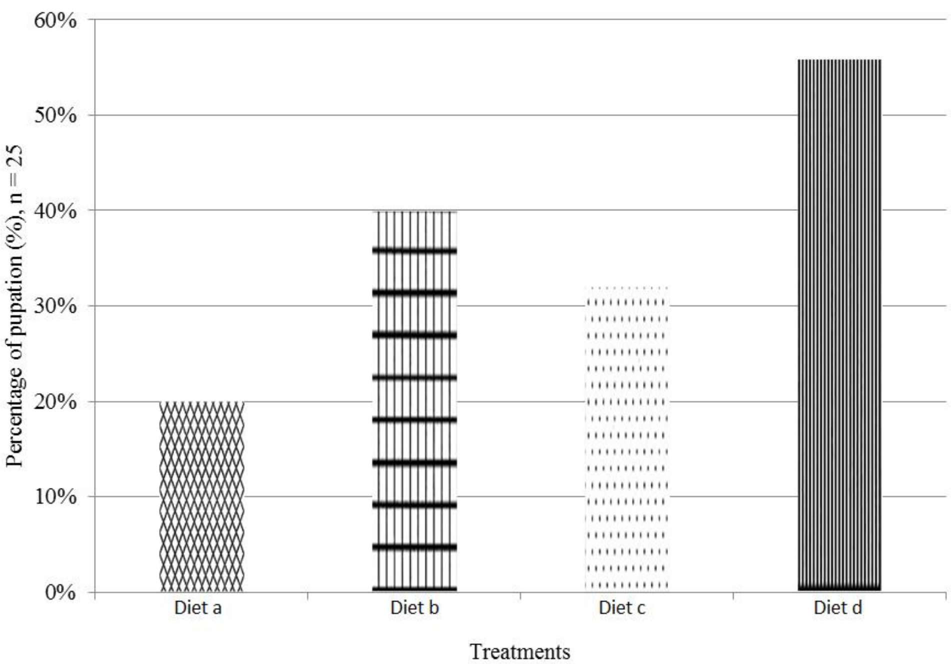


Figure 2 Percentage pupation of larvae *Elaeidobius kamerunicus* in different treatments

Figure 3 shows the percentage of emerging adults from pupae of *E. kamerunicus* in four different diets. The percentage number of emerged adults at diets a, diet b, diet c and diet d were 16%, 24%, 32% and 56% respectively. The highest percentage number of emerged adults occurred at diet d which was 56%, followed by diet c with 32% and diet b with 24%. The least percentage number of emerging adults occurred at diet a with only 16%. From table 3, the mean day of survival times of larvae *E. kamerunicus* for diet a, diet b, diet c and diet d were 5.958, 6.417, 7.091 and 8.625, respectively.

| Treatment | Mean[a] | | | | Median | | | |
|---|---|---|---|---|---|---|---|---|
| | Estimate | Std. Error | 95% Confidence Interval | | Estimate | Std. Error | 95% Confidence Interval | |
| | | | Lower Bound | Upper Bound | | | Lower Bound | Upper Bound |
| Diet a | 5.958 | 0.591 | 4.800 | 7.117 | 5.000 | 0.700 | 3.628 | 6.372 |
| Diet b | 6.417 | 0.640 | 5.163 | 7.670 | 5.000 | 0.403 | 4.211 | 5.789 |
| Diet c | 7.091 | 0.717 | 5.685 | 8.497 | 6.000 | 0.670 | 4.687 | 7.313 |
| Diet d | 8.625 | 0.667 | 7.318 | 9.932 | 11.000 | 0.000 | | |
| Overall | 7.021 | 0.338 | 6.358 | 7.684 | 6.000 | 0.570 | 4.882 | 7.118 |

Note: [a] Estimation is limited to the largest survival time if it is censored.

Table 3. Means and Medians for Survival time of larvae *E. kamerunicus*


| Treatment | Mean[a] | | | | Median | | | |
|---|---|---|---|---|---|---|---|---|
| | Estimate | Std. Error | 95% Confidence Interval | | Estimate | Std. Error | 95% Confidence Interval | |
| | | | Lower Bound | Upper Bound | | | Lower Bound | Upper Bound |
| Diet a | 5.958 | 0.591 | 4.800 | 7.117 | 5.000 | 0.700 | 3.628 | 6.372 |
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| Diet c | 7.091 | 0.717 | 5.685 | 8.497 | 6.000 | 0.670 | 4.687 | 7.313 |
| Diet d | 8.625 | 0.667 | 7.318 | 9.932 | 11.000 | 0.000 | | |
| Overall | 7.021 | 0.338 | 6.358 | 7.684 | 6.000 | 0.570 | 4.882 | 7.118 |

Note: [a] Estimation is limited to the largest survival time if it is censored.


## Comparison of Survival Rate of Larvae *E. Kamerunicus* Between Different Artificial Diets Under Laboratory Condition

### *Survival curve between treatment period and the number of survived larvae of E. kamerunicus*

Figure 4 shows trends of survival rate number of larvae *E. kamerunicus* at diet a, diet b and diet c decreased rapidly throughout the treatment period. However, the number of survived *E. kamerunicus* on diet d only slightly decreased throughout the treatment period. The number of development time of *E. kamerunicus* for different treatment was 11 days. The number of weevil *E. kamerunicus* increased significantly as the number of inoculated larvae increased, however the survival rate of *E. kamerunicus* significantly decreased. Therefore, in this experiment, the intersection of the regression curve of the survival rate, $y=-2.654x+28.6$ ($R^2=0.982$) was at 25 larvae placed in diet a when x considered as 0.

For diet b, the intersection of the regression curve of the survival rate, y=-2.430x+28.26 ($R^2$=0.946) while in diet c the intersection of the regression curve of the survival rate, y=-1.957x+25.46 ($R^2$=0.957). However, the intersection of the regression curve of the survival rate, y=-1.224x+26.13 ($r^2$=0.978) was at 25 larvae inoculated in diet d when x considered as 0. Thus, it seems reasonable to use 25 larvae since the number of survival weevils of *E. kamerunicus* at 25 larvae do not differ significantly from their respective maximum.

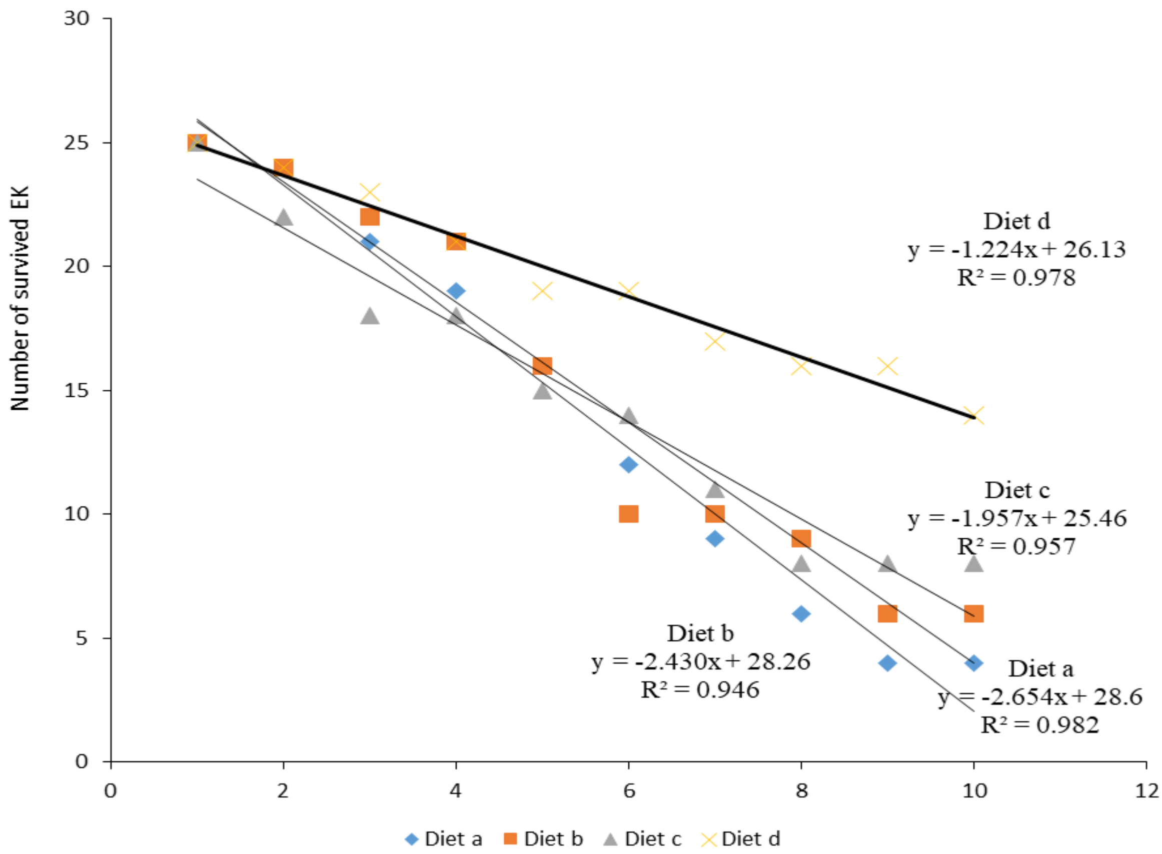


Figure 4. Survival rate between the treatment period and the number of survived larvae of *Elaeidobius kamerunicus*.

***Survival Curve of Larvae E. kamerunicus Tested on Different Artificial Diet***

In Fig. 5, Kaplan-Meier statistical analysis did not show any significant difference in the length of larval development among the diet (Chi-square=9.479; df=3; P>0.05). The mean ± SD number for survival time of larvae *E. kamerunicus* were 5.958± 0.591, 6.417±0.640, 7.091±0.717 and 8.625±0.667 for diet a, diet b, diet c and diet d respectively while the medium number of survival time of larval period were 6.372±0.700 for diet a, 5.789±0.403 for diet b, and 7.313±0.670 for diet c. Kaplan-Meier survival curves was plotted from the data showed minimum survival rate resulted at diet a while the maximum survival rate resulted at diet d. Observed population survived up to 10-11 days in all artificial diet.

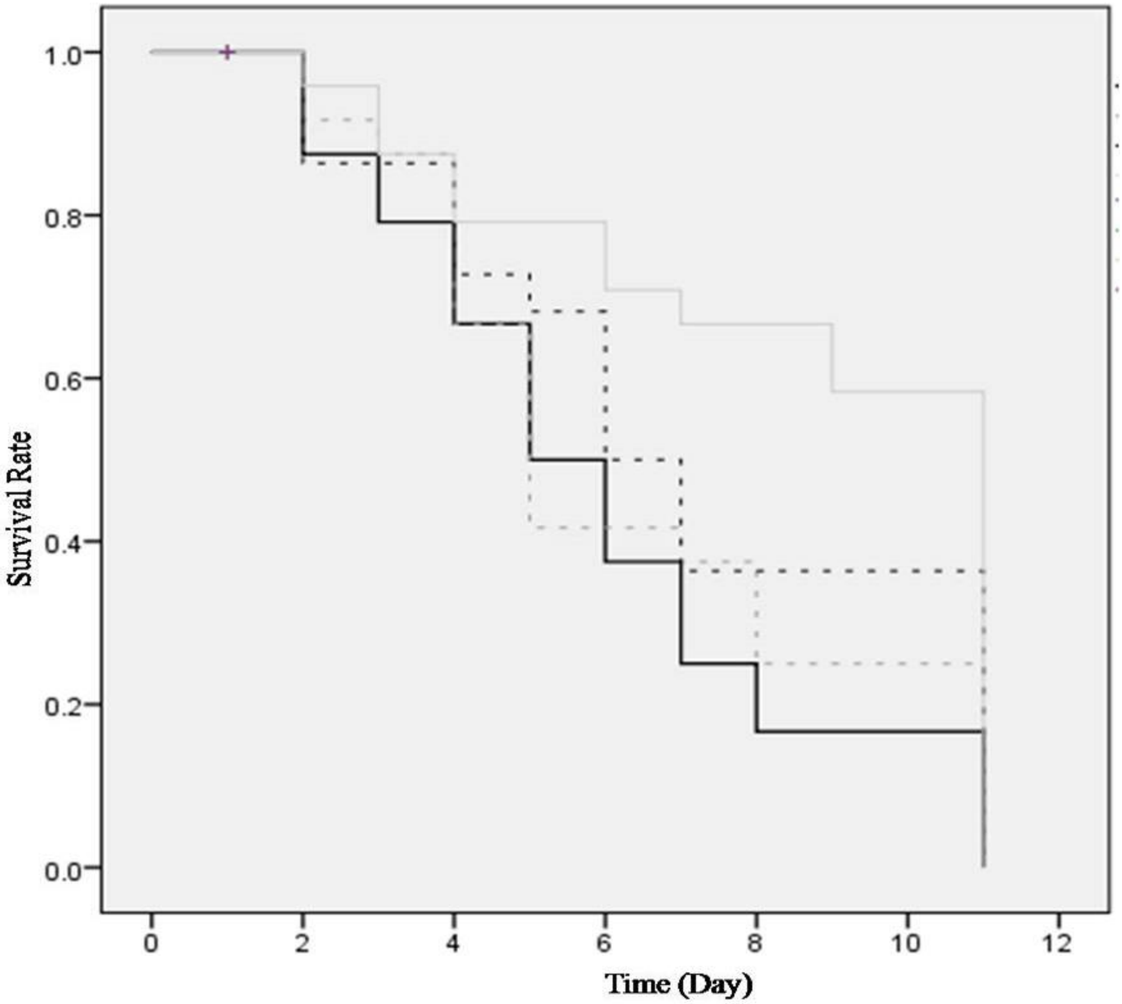


Figure 5. Kaplan-Meier survival curves of *Elaeidobius kamerunicus* larvae on different diet**.**
Note: black and solid: diet a; grey and broken: diet b; black and broken: diet c; grey and solid: diet d.

Table 4. Overall Comparisons of survival larvae of *E. kamerunicus* tested on different diet

| | Chi-Square | df | Sig. |
|---|---|---|---|
| Log Rank (Mantel-Cox) | 9.479 | 3 | 0.024 |

Note: Test of equality of survival distributions for the different levels of treatment.

Development of larvae *E. kamerunicus* at 25±0.1ºC tested with four different artificial larval diets resulted on different number of pupations ± standard deviation (%) and number of emerged adult ± standard deviation (%). As different ingredient used in artificial diets, different value of the development of larvae *E. kamerunicus.* The number of pupation ± SD (%) for diet a was 20±0.408, diet b was 40±0.500, diet c 32±0.476 and diet d 56±0.507 while the number of emerged adult ± SD (%) for diet a was 16 ± 0.374, diet b was 24±0.436, diet c was 32±0.476 and diet d was 56 ± 0.507. However, the day of emerge day (mean) also differs according to the different ingredient tested on the larvae *E. kamerunicus.*

Table 5. Development of larvae *Elaeidobius kamerunicus* at 25 ± 0.1ºC on four different artificial larval diets.

| | Artificial diet | | | |
|---|---|---|---|---|
| | a | b | c | d |
| No. of inoculated larvae | 25 | 25 | 25 | 25 |
| Mean of pupation ± SD (%) | 20 ± 0.408 | 40 ± 0.500 | 32 ± 0.476 | 56 ± 0.507 |
| Mean of emerged adults ± SD (%) | 16 ± 0.374 | 24 ± 0.436 | 32 ± 0.476 | 56 ± 0.507 |
| Day of adult emergence (mean) | 10.75 | 6.67 | 6.75 | 9.21 |

## DISCUSSION

Artificial diet is the most important factor in rearing insects while the natural diet that the insect consumes in its natural environment is ideal. However, it may be difficult to supply throughout the year, and its quality may vary according to seasonal changes such as during the rainy season where the natural diet in the natural habitat may affected by the fungus quickly. An artificial diet that can be maintained and supplied in a simple manner is desirable for stable mass rearing especially under laboratory condition. For example, a meridic diet for laboratory rearing of red palm weevil, *Rhynchophorus ferrugineus* (El-Shafie et al. 2011).

Artificial diet has been prepared and developed for other in weevil such as red palm weevil (Alarcòn et al. 2002), the Indian sweet potato weevil, *Euscepes Postfasciatus* (Fairmaire) (Shimoji & Kohama 1995), the *Oxyops vitiosa*, the *Diaprepes abbreviates* (Lapointe et al. 2010), and the Greater Wax Moth, *Galleria mellonella* L. (Metwally et al. 2012). One of the most common example of developed artificial diet which already developed and established since year of 1970s until this recent year in 2000s was the artificial diet of red palm weevil which continuously improved and contributed to the success of mass rearing of the weevil in laboratory condition with a food-fiber pupation technique which developed with 100% pupation efficiency (El-Shafie et al. 2011).

In this study, the larvae pupation and adult emerging of *E. kamerunicus* observation tested on four different artificial diets were done for 10-11 days. Comparison of larvae development of *E. kamerunicus* between four different constituents of diets have been carried out under laboratory condition. The measures taken in this experiment was to maintain the larvae survival until the emerged adults of *E. kamerunicus* on an artificial diet. Table 3 shows analysis of diets.

Our results showed that there were no obvious differences in number of pupations, emerging adults and in the days of adults emerging between the different tested artificial diets except for diet d which has slightly higher emerged adults compared to other diet. The percentage emerged adults which was 16% decrease from the 20% of pupation in tested diet a similar as diet b where the percentage of emerged adults decrease from the 40% of pupation to 24% of emerged adults due to unclear reasons. In practical artificial rearing, pupation, emerged adults and survival rate were important parameters that minor differences in size between adults reared on different constituents of artificial diets.

The Kaplan-Meier survival curves of *E. kamerunicus* larvae on different diets shows the value of log rank (Mantel-Cox) (Chi-square=9.479; df=3; P>0.05). The medium number of days of larval period was 6.372±0.700 for diet a, 5.789±0.403 for diet b, and 7.313±0.670 for diet c. Minimum survival rate resulted at diet a while the maximum survival rate resulted at diet d. this happen due to the different ingredient of artificial diet tested on the larvae of *E. kamerunicus.* The results indicate that the weevil larvae can be developed on the artificial diet with further studies on the longevity, fecundity and fertility of the *E. kamerunicus*.

The percentage number ± SD of pupation of larvae from artificial diet a, diet b, diet c and diet d were 20±0.408, 40±0.500, 32±0.476, 56±0.507 and 52±0.510 respectively and all the different was not significance (p > 0.05). The mean of developmental period from pupae to emerge adult from diet a, diet b, diet c and diet d were 10.75, 6.67, 6.75. 9.21 and 6.55 respectively. The percentage number ± SD of adults emerging from the artificial diet a, diet b, diet c and diet d were 16%±0.374, 24%±0.436, 32%±0.476 and 56 %±0.507 respectively. Significantly more adults emerged from diet d than from the other 3 diets (p>0.05). The results indicate that the weevil larvae can be developed on the artificial diet with further studies.

Our results showed that there were slightly differences in the number of pupations, emerging adults and the development day of adults emerging between diet a and diet b with the diet c and diet d with the ingredient of the artificial diets were different from diet a, diet b and diet c, diet d (Table 1). The results show that the diet d is superior to the other 3 diets in rearing with more than 50% of survive adult and pupae with better development day of emerging adults. The non-survived larvae of *E. kamerunicus* might cause by few factors such as the laboratory condition including the humidity and temperature, the unsuitable diet ingredient for the *E. kamerunicus* to survive and the unfavorable space used for the pupation and survival of larvae *E. kamerunicus.* In the findings of previous research by Ju et al. (2011) who stated that on suitable host plants, larvae may have fewer instars and thus its development can be shortened. The shorter life cycle on natural diet could indicate a smaller of larval instars.

However, the number and nature of molts can be frequently altered by external factors such as temperature, diet, and their interaction (Stamp 1990) and accordingly their number of larval instars can also be changed. There was variation occur in the literature concerning the number of larval instars of an insect that have been reared on different diets under laboratory condition from the previous research. For example, Ju et al. (2011) reported that there were eight larval instars of red palm weevil (*Rhynchophorus ferrugineus*) on Canary Island (*Phoenix canariensis*) and Washington palms (*Washingtonia* sp). Previous research reported nine larval instars of red palm weevil on Chusan palm (*Trachycarpus fortunei*), Pindo palm (*Butia capitata*), and Silver Date Palm (*Phoenix sylvestris*). Differently, there was also report on seven larval instars of red palm weevil on sugarcane while nine larval instars on Coconut Palm (*Cocos nucifera*) were recorded in Philippines by Jaya et al. (2000) and Viado and Bigornia (1949) respectively.

Previous research proved that the development of insect species may differ according to certain condition and different tested diet. This was supported by some researcher such as

E-Shafie et al. (2011), where the variation of life cycle and development day of weevil could be attributed to the rearing conditions and diet used by the researcher themselves. The differences in estimating the life parameters of weevil reared on artificial diet may have been occurred due to the ingredient of the diet and the rearing conditions (Joem & Behmer 1997).

Although there was some success in efforts to rear successive generations of economically important insects entirely on an artificial diet, in many cases there were also loss of both fitness and reproductive potential which cause longer development times and lower fecundity (Coudron et al. 2002). However, the successful pupation and emerged adults on artificial diet in this study might be useful to transfer the egg or larvae of *E. kamerunicus* from the origin habitat to new pollination place of oil palm plantation to increase the number of distributions of *E. kamerunicus.* The artificial diet of *E. kamerunicus* can also be used in applied study such as in previous study of Jalinas et al. (2015) under same condition.

Research conducted by Shimoji and Yamagishi (2004) succeeded in improving the number of surviving weevil *E. postfasciatus* per gram of diet by eight fold and in reducing the costs of diet materials to 30% from the conventional rearing method. Apart from that, the production of 1,000,000 weevils per week was achieved at a material cost of 187,500 yen. Therefore, the rearing method of insect such as *E. postfasciatus* in previous study using an improved artificial diet can also improve the survival rate of weevil besides reducing the materials costs. Further evaluation and study can be made to prove the survival rate between low-cost diet which were diet c and diet d with the high-cost diet of diet a and diet b.

## CONCLUSIONS

The rearing method for *E. kamerunicus* in this study using a modified artificial diets is successful in maintaining the highest emerged adult which is 56% out of 25 larvae on diet d. Additional directions of this study for *E. kamerunicus* need to be further research to improve the pupation rate and rate of the emerged adults. Although the suggested diet formulae of the host larvae did not show high significant differences in their survival rate, these diets differed in their cost, nutrient content and the texture of the diet. Diet d shows the most effective ones among the four diets for survival rate of *E. kamerunicus* larvae as artificial diet to maintain the larvae under laboratory condition.

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