Laboratory rearing of *Elaeidobius kamerunicus* Faust (Coleoptera: Curculionidae) pollinator of oil palm inflorescence in Malaysia

M H H Zulkefli¹, S Jamian¹2*, N A Adam¹, J Jalinas³ and M M Mohd Masri ⁴

¹Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
²Laboratory of Climate-Smart Food Crop Production, Institute of Tropical Agriculture and Food Security (ITAFoS), Universiti Putra Malaysia, Serdang Malaysia, 43400 Serdang, Selangor, Malaysia
³Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia
⁴Malaysian Palm Oil Board, 6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.

Corresponding author email: syari@upm.edu.my

Abstract. This study was conducted to evaluate laboratory rearing of *Elaeidobius kamerunicus* through observation of its life development and survivability on an artificial diet and anthesising male inflorescence of oil palm as the natural feed. The experiment was the three adopted diets compared with rearing on natural feed toward the survivability and life development of reared larvae *Elaeidobius kamerunicus*. The AMI of oil palm was the successful diet for the survival of weevil larvae with around 11 days to develop from egg to adult. The improved natural feed is calculated to have a longer time for 50% mortality than the first natural feed, where it had double the time requirement. Moreover, improving the natural feed rearing method had increased survivability from around 20% to almost 50%. Furthermore, the improved natural feed enables shorter life development at 10 days. Thus, this study provided the method for rearing *E. kamerunicus* through the natural feed for better key survival and management.

Keywords: Laboratory rearing, Oil palm, *Elaeidobius kamerunicus*, Life development

1. Introduction

*Elaeis guineensis* Jacq. is the most prominent agri-commodity in Malaysia, with 5.86 million hectares of cultivated area divided between plantation and smallholder [1] reduction by 0.6% in the previous year. The Malaysian export revenue from oil palm products in 2020 increased by 8.4% to RM73.25 billion compared to the 2019 export revenue [2].

Oil palm had both inflorescences in single palm, also known as monoecious plants [3]. During the early planting, the palm was reported as anemophilous wind [4]; later was documented as entomophilous during research in Cameroon in the late 70' [5]. Through the research, a pollinator species was introduced to Malaysian plantations to improve the low fruit set and high cost for assisted pollination [6]. The selected species was *Elaeidobius kamerunicus* based on its characteristic suitable for the tropical climate [7].
The oil palm’s anthesising male inflorescence (AMI) served as a feeding and repopulation site for the weevils [7]. This indicates the importance of the AMI to sustain the population of the weevil and is crucial for the development of fresh fruit bunches (FFB). The immature stage develops inside the male spikelet, specifically in the floret from egg to pupae, before emerging as an adult [7].

The obstacle to studying the biology of the weevil is mainly the development of the immature stage inside the spikelet, where it is difficult to monitor and can cause greater mortality if it is disturbed. Also, the AMI of oil palm is not readily available for daily use. Thus, the application of artificial diet is suitable to be used for this. First report of artificial diet by [8] documented successful rearing of the immature stage of E. kamerunicus. Nevertheless, the research lacks the comparison with the natural feed. The current report on the AMI of oil palm mainly is on estragole as an attractant for the weevil by [9, 11]. Thus, this study aims to determine the laboratory rearing of E. kamerunicus and its key survival.

2. Material and method

2.1. Collection and preparation of sampled anthesising male inflorescence of oil palm

Anthesising male inflorescence (AMI) of oil palm was collected from University Agriculture Park (Universiti Putra Malaysia) at (GPS: 2.988428, 101.722663), which was planted with Dura x Pisifera breed of fewer than 10 years old. Prior to collecting, the AMI was bagged with a muslin cloth to prevent infestation by insects until full anthesis. When achieved full anthesis, one male inflorescence was harvested and stored in a chiller at -5°C to be applied as natural feed, and another male inflorescence was set as a breeding ground for wild E. kamerunicus.

2.2. Elaeidobius kamerunicus larvae preparation.

The larvae of 3rd instar E. kamerunicus were prepared by removing the bagged full of AMI collected from oil palm. This allowed the wild adult E. kamerunicus to undergo mating for 24 to 48 hours and directly oviposit its egg inside the open floret. The method was modified from [12]. After the mating period, the whole inflorescence was harvested and stored in the laboratory to collect the larvae post 3 to 4 days incubated inside the spikelet to allow 3rd instar larvae to develop.

2.3. Study on laboratory rearing of E. kamerunicus

2.3.1. Preparation of artificial diet. The ingredients utilised were agar (Sigma), sucrose (Sigma), Vanderzant vitamin mixture for insects (Sigma-Aldrich), myo-inositol (Sigma), choline chloride (Sigma), Wesson salt mixture (MP Biomedicals), soy protein isolate (MP Biomedicals), cholesterol (Sigma), L-ascorbic acid (MP Biomedicals), potassium sorbate (Sigma-Aldrich), linseed oil (Sigma-Aldrich), soybean oil (Sigma), L-proline (MP Biomedicals) and 95% ethanol (Hamburg). The formulation of artificial diet is shown in Table 1 adopted from diet Anthonomus grandis [13] and Anthonomus tenebrosus [14]. The selection of diet from Anthonomus sp. based on it similar feeding of plant tissue based on a report by [15, 16] and similar size between E. kamerunicus and Anthonomus sp.

The preparation of artificial diet following [14]. The agar was dissolved in distilled water, then the mixture was boiled for a minute before being cooled to 75 °C. Finally, all the other ingredient was added. Inhibitor mixture 1:10 ratio of (10 g potassium sorbate to 100 ml of 95% ethyl alcohol) was prepared and added at 1.25 ml concentration/ 100 g of diet. Each rearing cup was poured with 10 ml of the diet then fed to the E. kamerunicus L3 instar larvae. Every three days, the diet was replaced with a new diet. The artificial diet (Diet 2 and Diet 3) was prepared following Diet 1 with pulverised freeze-dried AMI of oil palm and other ingredients listed in Table 1.
Table 1. Composition of three types of artificial diet formulation.

| Ingredients             | Diet 1  | Diet 2  | Diet 3  |
|-------------------------|---------|---------|---------|
| Agar                    | 3 g     | 3 g     | 3 g     |
| Water                   | 160 ml  | 160 ml  | 160 ml  |
| Sucrose                 | 8 g     | 8 g     | 8 g     |
| Vanderzant vitamins     | 0.02 g  | 0.02 g  | 0.02 g  |
| Inositol                | 0.04 g  | 0.04 g  | 0.04 g  |
| Choline chloride        | 0.10 g  | 0.10 g  | 0.10 g  |
| Wesson salt mixture     | 1.34 g  | 1.34 g  | 1.34 g  |
| Soy protein isolate     | 5 g     | 5 g     | 5 g     |
| Cholesterol             | 0.10 g  | 0.10 g  | 0.10 g  |
| L-ascorbic acid         | 0.40 g  | 0.40 g  | 0.40 g  |
| Male oil palm spikelet  | 0 g     | 0.18 g  | 0.18 g  |
| Lipids                  | 0 ml    | 0 ml    | 1.28 ml |
| L-proline               | 0 g     | 0 g     | 2 g     |
| Inhibitor mixture       | 2.50 ml | 2.50 ml | 2.50 ml |

2.3.2. Preparation of natural feed. The AMI of oil palm was used as the natural feed. Firstly, the natural feed was cut into a 4 cm long transverse section of the spikelet. Then each small section was placed in the rearing cup to feed the L3 instar larvae of *E. kamerunicus*. Every three-consecutive days, the natural feed was changed with a new section to prevent fungal infestation on the old spikelet. The natural feed was used as a comparison with the adopted artificial diet towards the growth development of *E. kamerunicus*.

2.4. Statistical analysis

The growth development of *E. kamerunicus* on artificial diet and natural feed observed was the survivability and life development (egg to adult). The experimental design implemented was a completely randomized design with n= 60 samples representing each diet's replication. The daily survivability data were analysed using correlation and regression method. In addition, all the data were statistically analysed using the SAS 9.4 version software.

3. Results and discussion

3.1. Comparison of laboratory rearing of *E. kamerunicus* in artificial diet and natural feed

3.1.1. Survivability of laboratory-reared *E. kamerunicus*. The daily survivability of the L3 larvae *E. kamerunicus* inoculated in the artificial diet against the natural feed of AMI of oil palm was presented in Figure 1. The correlation analysis recorded highly significant interaction with a negative and intermediate correlation between the hour exposed to the diet towards the survivability of the larvae in each diet applied. The result tabulated was Diet 1 (r=-0.4959, P<0.0001), Diet 2 (r=-0.4784, P<0.0001), Diet 3 (r=-0.5035, P<0.0001) and Natural Feed (r=-0.3796, P<0.0001).

The linear regression models were presented in figure 1 for all treatments. The linear regression for Natural feed indicates that when 50% survivability was expected, around 97 hours after exposure is needed. In comparison, the artificial diet required almost 63 hours for Diet 1, 74 hours for Diet 2, and 87 hours for Diet 3. This indicates the artificial diet required a shorter time to cause 50% mortality than the natural feed. All the artificial diets had a steeper incline in the linear model, showing faster mortality. Moreover, the final assessment reported that only natural feed caused adult emergence.
Figure 1. The survivability of inoculated larvae *E. kamerunicus* across hours exposed to diet.

The high mortality recorded on the natural feed need to be improvised to reflect a better diet for sustaining the weevil population. Thus, a new analysis was conducted using the improved natural feed. The improved rearing method was that the larvae had been kept in the same spikelet throughout the whole inoculation process. Moreover, the rearing cup was always kept sterile to prevent fungal infection on the spikelet. The correlation analysis for the improved natural feed was at \( r = -0.4736, P<0.0001 \) recorded highly significant interaction with a negative and intermediate correlation between the hour exposed to the diet towards the survivability of the larvae. The linear regression in figure 1 shows that when 50% survivability was expected, the estimated hour to achieve it was 194 hours. This indicates double the required time as the first natural feed. Besides that, this exhibit suitability as the rearing method for the natural feed.

The AMI of oil palm was analysed for its nutrient composition by [17] through proximate analysis at 4.1g/100g protein, 0.4g/100g total fat, 20.3g/100g total carbohydrate, and the highest composition of moisture content at almost 75%. Applying an artificial diet for rearing the weevil lacks the essential need for the weevil to emerge as an adult based on its low nutrient content. The artificial diet utilising the main sources of protein and lipid from soy protein isolate and carbohydrate from the sucrose in table 1 was below the value of the natural feed in g/100g based on a report by [17]. The reported data were compared with data from [18] and [19], where it calculated around 60% of protein, 20% total fat, and 22% total carbohydrate for the artificial diet compared to the AMI of oil palm. Thus, indicate insufficient nutrients to support the larvae *E. kamerunicus* in the adopted diet. On the contrary, [8] reported that the successful rearing of *E. kamerunicus* larvae using a low-cost diet with main constituents (honey, wheat flour, milk powder, yeast, and cornflour) resulted in 56% of adult emergence. The adopted artificial diet needs to be improved in terms of the nutrient content for its suitability as rearing feed for the weevil as of the low-cost diet [8].

3.1.2. Life development of reared *E. kamerunicus* on natural feed.

The life development of the laboratory-reared *E. kamerunicus* on AMI of oil palm recorded around 11 days to complete the life development from egg to adult. The summary of the life development was summarised in figure 2. The minimum day recorded from 13 sampled (7 female, 6 male) of emerged *E. kamerunicus* adults was 9 days and a maximum of 12 days. The pupation period was around two to four days.
Figure 2. Summary of life development of reared *E. kamerunicus* on natural feed (AMI).

Figure 3. Summary of life development of reared *E. kamerunicus* on improved natural feed (AMI).

On the contrary, the improved natural feed enables *E. kamerunicus* to exhibit faster life development at average 10 days in figure 3 than the first natural feed in figure 2. The total life development was between 9 to 13 days from 24 samples (10 female, 14 male). Despite it had a longer pupation period between one to four days compared to the first natural feed. When the life development was compared with the summarized report of *E. kamerunicus* life cycle by [20], it was among the fastest development of *E. kamerunicus*. The summarized report of [20] stated that at 19 days in Cameroon [7], 15 days reared inside a laboratory in Malaysia [12], 7 to 14 days in a Malaysian quarantine facility during the early introduction to Malaysia [21], 15 days [22] and 14 days [23] in Ivory Coast, and 12 days [24] in Indonesia. The climate can cause the variation and possibly conclude by [25] of temperature where it stated that higher temperature induced faster development. The average temperature for the first natural feed was around 28.8°C ± 0.27 causing around 11 days development and improved natural feed at 31.5°C ± 0.17 enable around 10 days development.

4. Conclusion
The AMI of oil is crucial for the growth development of *E. kamerunicus*. This finding is key to developing a suitable artificial diet that can sustain and support the whole development of *E. kamerunicus* as there is a lack of reports on the application of artificial diet for *E. kamerunicus*. Nevertheless, further analysis is needed to improve nutrients on the artificial diet and ultimately support the survival of *E. kamerunicus* in laboratory-reared conditions.

Acknowledgments
The Universiti Putra Malaysia funded the project under the Putra Grant (GP-IPM/2020/9687000).

References
[1] MPOB 2021 Oil palm planted area 2020 (https://bepi.mpob.gov.my/images/area/2020/Area_summary.pdf)
[2] Ahmad Parveez G K, Ahmad Tarmizi A H, Sundram S, Soh K L, Ong-Abdullah M, Poo Palam K D, Mohamed Salleh K, Mohd Ishak S and Idris Z 2021 Oil Palm Economic Performance in Malaysia and R&D Progress in 2020 *J. Oil Palm Res.* **33** 181–214
[3] Kushairi A, Parveez GKA and Kamarudin N 2019 Perusahaan sawit di Malaysia: Satu panduan. (Edisi Keem) (Selangor, Malaysia: Lembaga Minyak Sawit Malaysia)

[4] Hardon J J and Turner P D 1967 Observations on natural pollination in commercial plantings of oil palm (Elaeis guineensis) in Malaya Exp. Agric. 3 105–16

[5] Syed R A 1979 Studies on oil palm pollination by insects Bull. Entomol. Res. 69 213–24

[6] Syed R A, Law IH and Corley RHV 1982 Insect pollination of oil palm: introduction, establishment and pollinating efficiency of Elaeodobius kamerunicus in Malaysia The Planter Kuala Lumpur 58 547–61

[7] Syed R A 1981 Insect pollination of oil palm: feasibility of introducing Elaeodobius spp. into Malaysia Oil Palm News 25 2–16

[8] Sarah Najihah M Z, Rosha R, Mohamad Haris H and Johari J 2019 Survival rate and development of larvae Elaeodobius kamerunicus (Coleoptera: Curculionidae) on artificial diets Serangga 24 126–41

[9] Opute F I 1975 Identification of p-methoxyallylbenzene in the pollen of the oil palm Elaeis guineensis Jacq. J Exp. Bot. 26 619–23

[10] Lajis N H, Hussein M Y and Toia R F 1985 Exraction and identification of the main compound present in Elaeis guineensis flower volatiles Pertanika 8 105–8

[11] Muhamad Fahmi M H, Ahmad Buhkary A K, Norma H, Idris AB 2016 Analysis of volatile organic compound from Elaeis guineensis inflorescences planted on different soil types in Malaysia AIP Conference Proceedings p 1–5

[12] Hussein M Y and Rahman W H A 1991 Life tables for Elaeodobius kamerunicus Faust (Coleoptera : Curculionidae) in oil palm The Planter Kuala Lumpur 67 3–8

[13] Earle N W, Walker A B and Burks M L 1966 An artificial diet for the boll weevil , Anthonomus grandis (Coleoptera : Curculionidae), based on the analysis of amino acids in cotton squares 1’ 2 Am. Entomol. Soc. Am. 59 664–9

[14] Davis B J 2007 Evaluation of artificial diets for rearing Anthonomus tenebrosus (Coleoptera: Curculionidae), a potential biological control agent of tropical soda apple, Solanum viarum. (Florida: Thesis of University of Florida)

[15] Medal J, Bustamante N, Bredow E, Pedrosa H, Overholt W, Díaz R and Cuda J 2011 Host Specificity of Anthonomus tenebrosus (Coleoptera: Curculionidae), A Potential Biological Control Agent of Tropical Soda Apple (Solanaceae) in Florida Florida Entomol. 94 214–25

[16] Jeger M, Bragard C, Caffier D, Candresse T, Chatzivassiliou E, Dehnen-Schmutz K, Gilioli G, Gregoire J C, Jaques Miret J A, Navarro M N, Niere B, Parnell S, Potting R, Rafoss T, Rossi V, Urek G, Van Bruggen A, Van der Werf W, West J, Winter S, Gardi C, Bergeretti F and MacLeod A 2017 Pest categorisation of Anthonomus grandis EFSA J. 15 1–21

[17] Zulkefli M H H, Jamian S, Adam N A, Jalinas J and Mohd Masri M M 2021 Evaluation of artificial diet on growth development of Elaeodobius kamerunicus Faust (Coleoptera: Curculionidae) Serangga 26 132–50

[18] USDA 2019 Food data central (Soy protein isolate) (https://fdc.nal.usda.gov/fdc-app.html#food-details/174276/nutrients)

[19] Sigma-Aldrich 2021 Sucrose for molecular biology, ≥99.5% (GC) (https://www.sigmaaldrich.com/MY/en/product/sigma/s0389?context=product#)

[20] Zulkefli M H H, Jamian S, Adam N A, Jalinas J, Mohamad SA and Mohd Masri MM 2020 Beyond four decades of Elaeodobius kamerunicus Faust (Coleoptera : Curculionidae) in the Malaysian oil palm industry : a review J. Trop. Ecol. 36 282–92

[21] Karim Z A 1982 Screening of Elaeodobius kamerunicus Faust in Malaysia (Kuala Lumpur: Crop Protection Branch, Department of Agriculture)

[22] Maraiu D, Houssou M, Lecoustre R and Ndiguib B 1991 Insectes pollinisateurs du palmier à huile et taux de nouaison en Afrique de l’Ouest Oléagineux 46 43–51

[23] Tuo Y, Koua H K, Hala N 2011 Biology of Elaeodobius kamerunicus and Elaeodobius plagiatus (Coleoptera: Curculionidae) main pollinators of oil palm in West Africa Eur. J. Sci. Res. 49
426–32

[24] Girsang R J, Tobing M C and Pangestuningsih Y 2017 Biologi serangga penyerbuk Elaeidobius kamerunicus (Coleoptera: Curculionidae) setelah 33 tahun diintroduksi di Sumatera Utara J. Agroekoteknologi FP USU 5 348–54

[25] Herlinda S, Pujiasutti Y, Adam T and Thalib R 2006 Daur hidup kumbang penyerbuk Elaeidobius kamerunicus Faust (Coleoptera: Curculionidae) bunga kelapa sawit (Elaeis guineensis Jacq) Agria. 3 10–2