

Original Article

## Impact of artificial diets on the biological and chemical properties of red palm weevil, *Rhynchophorus Ferrugineus* Olivier (Coleoptera:Curculionidae)

Impacto de dietas artificiais nas propriedades biológicas e químicas do caruncho da palmeira, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae)

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### Abstract

One of the most common palm pests is the red palm weevil, *Rhynchophorus ferrugineus* Olivier. Five artificial diets were evaluated to rear RPW, and to facilitate its behavioral and biological studies. The developmental of *R. ferrugineus* was studied on the tested artificial diets based on primarily composed of sugarcane stem pieces (1<sup>st</sup> diet; control), sweet pumpkin (2<sup>nd</sup> diet), ground sugarcane + sweet pumpkin (3<sup>rd</sup> diet), sweet potato (4<sup>th</sup> diet) and ground sugarcane + sweet potato (5<sup>th</sup> diet) under rearing room (28 ± 1 °C and 75 ± 5 RH%) at Faculty of Agriculture in Benha district. The biological aspects, pre-oviposition, oviposition, larval & pupal durations, the longevity of male and female adults and the generation period of *R. ferrugineus* have all been estimated. Moreover, the impact of these artificial diets on the quantities of total proteins, carbohydrates and lipids were determined in the full-grown larvae. Obtained results showed that the insect manifested the shortest larval and pupal periods and the longest longevity for males and females when rearing of *R. ferrugineus* took place on an artificial diet based on sweet potato as a successful and economical for mass rearing of the red palm weevil under laboratory conditions. Larvae fed on diet 4 (sweet potato) was exhibited the shortest duration (114.80 days), while 1<sup>st</sup> diet (Sugarcane stem pieces) was recorded the longest period (125.00 ± days). Similarly for pupation and both male and female longevity, the shortest periods resulted from larvae feeding on 4<sup>th</sup> diet (14.65, 49.40 and 48.0 days, respectively). Contrariwise, the longest pupal period was reported with 5<sup>th</sup> diet (15.70 days). The highest of eggs hatchability % (96.53%) resulted from the rearing of *R. ferrugineus* larvae on the 4<sup>th</sup> diet. Chemical analyses of the full-grown larva were indicated that the highest contents of total carbohydrates (45.57 µg/g), lipids (28.46 µg/g) and proteins (20.01µg/g) were obtained in case of larvae reared on 1<sup>st</sup> and 4<sup>th</sup> diets, respectively. Consequently, the development of *R. ferrugineus* stages was slightly lower on both 1<sup>st</sup> and 4<sup>th</sup> artificial diets. So that, both of them were a successful and economic artificial diet for RPW development under laboratory conditions. Therefore, this study added valuable alternative ingredients to prepare more practical and economical feeding substrate for weevil rearing to help for proposal of its management and conservation in palm plantations.

**Keywords:** artificial diets, biological aspects, chemical contents, *Rhynchophorus ferrugineus*, RPW.

### Resumo

Uma das pragas mais comuns das palmeiras é o gorgulho vermelho da palmeira, *Rhynchophorus ferrugineus* Olivier. Cinco dietas artificiais foram avaliadas para criar RPW e facilitar seus estudos comportamentais e biológicos. O desenvolvimento de *R. ferrugineus* foi estudado nas dietas artificiais testadas baseadas principalmente em pedaços de caule de cana-de-açúcar (1<sup>a</sup> dieta; controle), abóbora-doce (2<sup>a</sup> dieta), cana-de-açúcar + abóbora-doce (3<sup>a</sup> dieta), batata-doce (4<sup>a</sup> dieta) e cana-de-açúcar moída + batata-doce (5<sup>a</sup> dieta) em sala de criação (28 ± 1 °C e 75 ± 5 UR%) na Faculdade de Agricultura do distrito de Benha. Os aspectos biológicos, pré-oviposição, oviposição, durações de larvas e pupas, a longevidade de machos e fêmeas adultos e o período de geração de *R. ferrugineus* foram todos estimados. Além disso, o impacto dessas dietas artificiais nas quantidades de proteínas totais, carboidratos e lipídios foi determinado nas larvas adultas. Os resultados obtidos mostraram que o inseto manifestou os menores períodos larval e pupal e a maior longevidade para machos e fêmeas quando a criação de *R. ferrugineus* ocorreu em uma dieta artificial à base de batata-doce como uma forma bem-sucedida e econômica para a criação massal do gorgulho vermelho da palmeira em condições de laboratório. Larvas alimentadas com a dieta 4 (batata-doce) foram as que apresentaram a menor duração (114,80 dias), enquanto a 1<sup>a</sup> dieta (pedaços de caule de cana-de-açúcar) foi a que apresentou o maior período (125,00 ± dias). Da mesma forma para a pupação e longevidade de machos e fêmeas, os períodos mais curtos resultaram da alimentação das larvas na 4<sup>a</sup> dieta (14,65, 49,40 e 48,0 dias, respectivamente). Ao contrário, o período pupal mais longo foi relatado com a 5<sup>a</sup> dieta (15,70 dias). A maior

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Received: May 28, 2022 – Accepted: July 25, 2022



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porcentagem de eclodibilidade dos ovos (96,53%) resultou da criação de larvas de *R. ferrugineus* na 4ª dieta. As análises químicas das larvas adultas indicaram que os maiores teores de carboidratos totais (45,57 µg/g), lipídios (28,46 µg/g) e proteínas (20,01µg/g) foram obtidos no caso de larvas criadas na 1ª e 4ª dietas, respectivamente. Consequentemente, o desenvolvimento dos estádios de *R. ferrugineus* foi ligeiramente menor tanto na 1ª quanto na 4ª dieta artificial. Assim, ambas foram dietas artificiais bem-sucedida e econômica para o desenvolvimento de RPW em condições de laboratório. Portanto, este estudo adicionou ingredientes alternativos valiosos para preparar substrato alimentar mais prático e econômico para a criação do gorgulho para auxiliar na proposta de seu manejo e conservação em plantações de palmeiras.

**Palavras-chave:** dietas artificiais, aspectos biológicos, conteúdos químicos, *Rhynchophorus ferrugineus*, RPW.

## 1. Introduction

Date palm, *Phoenix dactylifera* L. (Arecaceae: Arecaceae) is the most common and widely cultivated plant in the arid and semiarid regions of the Arabian Peninsula, Middle East, and North Africa for about 5000 years. The fruit dates of the date palm are very rich in carbohydrates, vitamins, fiber, fat, protein, and minerals (Purseglove, 1972; Jones, 1995). The date palm crops are under serious threats by the direct attack of red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), which is considered a key pest of date palm in these regions (Faleiro, 2006). This insect was reportedly attacking extensive host ranges over 26 palm species belonging 16 genera (Morici, 1998; Malumphy and Moran, 2007) and is able to adapt to live in a wide variety of environmental conditions, and females lay eggs in the trunk of palm trees where larvae feed on the soft part and the apical growing causing stunted growth and death of the palm trees (Murphy and Briscoe, 1999). In the past 30 years, *R. ferrugineus* was highly destructive pest to all types of palms, has rapidly spread worldwide (Kurdi et al., 2021). Detection of the beginning infestation of RPW is highly challenging because symptoms are not visible until the badly damaged and started to die of the palm tree is inevitable (Faleiro, 2006; Kurdi et al., 2021).

Laboratory rearing of insect pests maintained the purity, age and sex-based selection is very important for conducting lab. and field tests for suggesting and improving the control strategies. The knowledge about insect biology, behavior, ecology and availability of various stages in bulk amounts facilitates keeping many factors unbiased (Shahina et al., 2009). Mass rearing of *R. ferrugineus* on sugarcane, banana and date palm under laboratory conditions kept RPW reared on sugarcane to be used as control before rearing it on other artificial diets (Kaakeh et al., 2001).

Additionally, artificial diets based on natural agents, banana, sugarcane, squash fruit, apple and palm crown were carried out to study the biology of RPW by Salama et al. (2009). They found that the generation time was the shortest duration on palm crown, followed by banana and then squash fruit and apple, but it was longer on sugarcane. Also, The RPW life cycle parameters were determined by Francardi and Benvenuti (2010) in the laboratory using the four artificial diets of previously authors but with the replacement of some structures, i.e. a mixture of unidentified cereals with buckwheat alone, molasses with polyfloral honey, an alternative diets, respectively composed of honey, dried coconut cake and oat or commercial apple mousse. Moreover, Mahmoud et al. (2015) studied the effect of diets (sugar cane slices, semi-artificial diet, and

palm heart) on biological aspects of the red palm weevil under laboratory conditions.

To Conduct the biological and control studies, a large number of *R. ferrugineus* stages requires. Therefore, mass rearing is needed to implement this issue. Rearing *R. ferrugineus* on date palm tissues could be unavailable and expensive, so, an artificial diet is needed. An ideal diet for mass rearing of red palm weevils should provide all the required nutrients for larval feeding in a sufficient quantity and be easy to prepare and store for an extended period. Artificial diets for mass-rearing, also, have to be inexpensive and produce an average yield of acceptable adults. The presented study aimed to develop artificial diets as a substitution for date palm tissues for laboratory rearing of *R. ferrugineus*.

## 2. Materials and Methods

### 2.1. Biological aspects of *R. Ferrugineus* reared on different artificial diets

#### 2.1.1. Insect culture

Male and female adults of the red palm weevil, *R. ferrugineus* were collected from date palm groves at Qassasin district, Ismailia Governorate, in 2020, and then used as the initial individuals for this insect culture. The RPW stages were reared in a rearing room at  $28 \pm 1$  °C and  $75 \pm 5\%$  in laboratories of Agriculture Faculty, Benha University.

#### 2.1.2. Artificial diets used for *R. ferrugineus* rearing (Figure 1)

#### 2.1.3. Durations of immature stages

##### 2.1.3.1. Egg incubation period

Twenty couples (male & female) of the freshly emerged *R. ferrugineus* adults were collected and kept in containers and fed on the tested diets. Adults were left for one week to ensure the copulation occurred. Deposited eggs were removed daily by peeling the fibrous tissues and picking up eggs using a camel's hairbrush. To avoid the dryness of eggs, these were placed on wet filter paper (10 cm diameter) and monitored until hatching.

##### 2.1.3.2. Larval and pupal stages

Freshly emerged larvae were gently removed and distributed in glass bottles (5 × 10 cm; Figure 2) containing different diets. Larvae were daily observed until reached
















Diets	Component				
1 <sup>st</sup> Diet	Sugarcane stem pieces (Control)		 1 <sup>st</sup> Diet	 1 <sup>st</sup> Diet +Larvae	 Last larval instar
2 <sup>nd</sup> Diet	Components	Amount	 2 <sup>nd</sup> Diet	 2 <sup>nd</sup> Diet +Larvae	 Last larval instar
	Sweet pumpkin	250 g			
	Agar	5 g			
	Dried active yeast	15 g			
	Sorbic acid	1.25 g			
	L- Ascorbic acid	2.50 g			
	Sodium benzoate	1.25 g			
	Distilled water	100-150 ml			
3 <sup>rd</sup> Diet	Components	Amount	 3 <sup>rd</sup> Diet	 3 <sup>rd</sup> Diet +Larvae	 Last larval instar
	Ground Sugarcane	125 g			
	Sweet pumpkin	125 g			
	Agar	5 g			
	Dried active yeast	15 g			
	Sorbic acid	1.25 g			
	L- Ascorbic acid	2.50 g			
	Sodium benzoate	1.25 g			
Distilled water	100-150 ml				
4 <sup>th</sup> Diet	Components	Amount	 4 <sup>th</sup> Diet	 4 <sup>th</sup> Diet +Larvae	 Last larval instar
	Sweet potato	250 g			
	Agar	5 g			
	Dried active yeast	15 g			
	Sorbic acid	1.25 g			
	L- Ascorbic acid	2.50 g			
	Sodium benzoate	1.25			
	Distilled water	100-150 ml			
5 <sup>th</sup> Diet	Components	Amount	 5 <sup>th</sup> Diet	 5 <sup>th</sup> Diet +Larvae	 Last larval instar
	Ground Sugarcane	125 g			
	Sweet potato	125 g			
	Agar	5 g			
	Dried active yeast	15 g			
	Sorbic acid	1.25 g			
	L- Ascorbic acid	2.50 g			
	Sodium benzoate	1.25 g			
Distilled water	100-150 ml				

Figure 1. Artificial diets used for rearing of *R. ferrugineus* larvae: 1<sup>st</sup> Diet, (Control); 2<sup>nd</sup> Diet; 3<sup>rd</sup> Diet; 4<sup>th</sup> Diet and 5<sup>th</sup> Diet.

their last instar. At this stage, the larvae were moved to plastic boxes covered with light-pored covers (25 × 20 × 10 cm; Figure 3) where pieces of palm fibers were added as suitable pupating substrates (Figure 4). The total larval period for the larvae reared on different diets was been recorded. The formed pupae were individually placed in

small cups (5 × 5 cm) covered with the pored lids and were observed until the adult emergence.

2.1.3.3. Adult stage

Every pair of emerged adults was kept in a cup (5 × 10 cm) containing small stem pieces of sugarcane. Adults





**Figure 2.** Small cups for young larvae.



**Figure 3.** Large containers for older larvae.



**Figure 4.** Suitable Container for pupation (Palm fibers).

were observed daily, and the sugarcane pieces were removed, and then kept in other cups, and replaced with new pieces whenever needed. The adults were left in the cups until mortality, and the longevity of males and females were estimated and recorded.

#### 2.1.3.4. Eggs productivity/female

The small stem pieces of sugarcane containing eggs were carefully dissected, after that, the deposited eggs were collected, counted and kept until the hatching as mentioned by Abdel-Hameid (2019).

#### 2.2. Biochemical assays on *R. Ferrugineus* reared on different artificial diets

The samples were prepared as described by Amin (1998). The larvae were homogenized in distilled water (50 µg/1 mL). Homogeneity was centrifuged at 8000 rpm for 15 minutes at 2 °C in a refrigerated centrifuge. The deposits were discarded and the supernatant (enzyme extract) could be stored for at least one week without significant loss in activity at -50 °C. For biochemical analyses, insects were homogenized in a chilled glass Teflon tissue homogenizer (ST - 2 Mechanic - Preczyina, Poland). The supernatants were kept in a deep freezer at -50 °C until used. A double beam UV spectrophotometer (Spectronic 1201, Milton Roy Co., USA) was used to measure the absorbance of colored substances or metabolic compounds. The different materials were used as Bovine albumin standard (from Stan bio laboratory, Texas, USA), Coomassie brilliant blue G-250 (from Sigma chemicals co.), P-nitro anisole (purity 97%) (Ubichem Ltd., Hampshire), Nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) (from BDH chemicals Ltd., Poole, England). However, the rest of the chemicals were used in this study from local commercial companies.

To study the biochemical assessment, The total lipids were estimated according to Knight et al. (1972) using a phosphor vanillin reagent. Additionally, the total carbohydrates were also determined by using the phenol-sulphuric acid reaction method as that applied by Dubois et al. (1956). Moreover, carbohydrates from insect tissues were extracted and prepared to assay according to Crompton and Birt (1967). The total proteins content was determined according Bradford method (Bradford, 1976).

#### 2.3. Statistical analysis

Obtained data were analyzed using ANOVA with three factors at a 0.05 significance level. Pairwise comparisons were performed using LSD for multiple comparisons. All statistical analyses were carried out using SPSS soft program.

### 3. Results

#### 3.1. Biological development of *R. Ferrugineus* reared on different artificial diets

##### 3.1.1. Eggs' incubation period

The eggs' hatching occurred at ranged between  $3.35 \pm 0.11$  and  $4.60 \pm 0.11$  days after the rearing on five

tested diets (Table 1). The obtained eggs after rearing on 4<sup>th</sup> diet manifested the shortest incubation period.

### 3.1.2. Effect of different diets on *R. ferrugineus* arval duration

Data presented in Table 1 cleared that the longest larval period of the first generation of RPW, was significantly, ( $125 \pm 5.08$  days) occurred when the insect was reared on sugarcane stem pieces (1<sup>st</sup> diet). contrariwise, this period non-significantly varied from 114.8 to 115.7 days when the rearing was took place on the remained four artificial diets (Table 1). The fastest larval development of *R. ferrugineus* has been recorded on the food source of 4<sup>th</sup> Diet.

### 3.1.3. Duration of the pupal stage

Statistical analysis of results showed that a significant difference was obtained between the pupal duration of the pupae after the rearing on the tested diets (Table 1). the shortest was nonsignificantly ( $14.65 \pm 0.13$  days in case of 4<sup>th</sup> diet) being shorter than those obtained after the rearing on 2<sup>nd</sup> diet ( $14.75 \pm 0.18$  days). On contrary, the longest pupal duration ( $15.70 \pm 0.15$  days) was obtained after the rearing occurred on 5<sup>th</sup> diet, and nonsignificantly followed, by those from the rearing on 3<sup>rd</sup> diet. During this period, the pupae remain in their cocoons until the adults' emergence. Palm fibers were placed to improve pupation because the full-grown larvae used these fibers to construct their cocoons (Figure 4).

### 3.1.4. Total developmental period

The total duration of immature stages was the longest ( $144.45 \pm 5.16$  days) after the rearing on the natural diet (sugarcane stem pieces) being significantly longer than those recorded from the remained diets (ranged from  $129.45 \pm 0.37 - 136 \pm 0.61$  days). The fastest immature development of *R. ferrugineus* has been obtained from the food source of 4<sup>th</sup> Diet, and the longest after rearing on 5<sup>th</sup> diet (Table 1).

## 3.2. Biological aspects of *R. ferrugineus* reared on different artificial diets

### 3.2.1. Adults' longevity

The obtained data in Table 2 showed that the rearing of RPW larvae on the different-tested diets had influenced on the longevity of the emerged adults. The type of larval diet provided for larval feeding manifested to have a significant effect on the longevity of the resultant males and females. The longest adult longevity ( $49.40 \pm 0.24$  and  $48.00 \pm 0.45$  days for males females, respectively) was observed after rearing on 4<sup>th</sup> diet. Data concerning the effect of the remaining 4 rearing diets on the adults' longevity indicated non-significant differences between them.

### 3.2.2. Sex ratio

Data presented in Table 2 showed the sex ratio among emerged adults (as a Female %). The sex ratio (♀:♂) was

**Table 1.** Effect of different diets on the developmental durations (Mean  $\pm$  SE) of *Rhynchophorus ferrugineus* stages at  $28 \pm 1$  °C and  $75 \pm 5\%$  RH.

Diets	Developmental time (days)			Total developmental period
	Egg' incubation	Larvae	Pupa	
1 <sup>st</sup> Diet	$4.30 \pm 0.11^a$	$125.00 \pm 5.08^a$	$15.15 \pm 0.11^b$	$144.45 \pm 5.16^a$
2 <sup>nd</sup> Diet	$4.40 \pm 0.11^a$	$114.90 \pm 0.34^b$	$14.75 \pm 0.18^c$	$133.85 \pm 0.33^b$
3 <sup>rd</sup> Diet	$4.30 \pm 0.11^a$	$115.45 \pm 0.41^b$	$15.35 \pm 0.11^{ab}$	$135.10 \pm 0.46^b$
4 <sup>th</sup> Diet	$3.35 \pm 0.11^b$	$114.80 \pm 0.33^b$	$14.65 \pm 0.13^c$	$129.45 \pm 0.37^b$
5 <sup>th</sup> Diet	$4.60 \pm 0.11^a$	$115.70 \pm 0.65^b$	$15.70 \pm 0.15^a$	$136.00 \pm 0.61^b$
LSD at 0.05	0.31	6.49	0.38	6.58

Values denoted by the same letter in the same column are non-significantly different at 0.05 level. SE = standard error; RH = relative humidity.

**Table 2.** Some biological aspects of *Rhynchophorus ferrugineus* after reared in their larval stage on different diets (Mean  $\pm$  SE).

Diet	No. of eggs/ female	No. of hatched eggs	% Hatching	Adult longevity (days)		Female%	Sex ratio	
				Female	Male		♀:♂	
1 <sup>st</sup> Diet	$202.00 \pm 5.83^b$	$193.20 \pm 4.87^b$	$95.69 \pm 0.39^{ab}$	$44.80 \pm 0.58^b$	$45.60 \pm 0.81^b$	50	1	1
2 <sup>nd</sup> Diet	$204.00 \pm 6.00^b$	$194.60 \pm 5.40^b$	$95.41 \pm 0.17^b$	$44.80 \pm 0.58^b$	$45.60 \pm 0.81^b$	50	1	1
3 <sup>rd</sup> Diet	$198.00 \pm 7.35^b$	$189.40 \pm 6.49^b$	$95.70 \pm 0.30^{ab}$	$44.80 \pm 0.58^b$	$45.60 \pm 0.81^b$	55	1.2	1
4 <sup>th</sup> Diet	$254.00 \pm 6.78^a$	$245.20 \pm 6.71^a$	$96.53 \pm 0.48^a$	$48.00 \pm 0.45^a$	$49.40 \pm 0.24^a$	55	1.2	1
5 <sup>th</sup> Diet	$218.00 \pm 8.00^b$	$208.60 \pm 7.85^b$	$95.67 \pm 0.12^{ab}$	$44.80 \pm 0.73^b$	$46.80 \pm 0.97^b$	45	0.8	1
LSD at 0.05	20.18	18.74	0.94	1.75	2.28			

Values signed by the same lowercase letter in the same column are non-significantly different at  $P > 0.05$ .

1: 1 after rearing on diets, 1& 2, 1.2: 1 with feeding on 3& 4 and 0.8: 1 on diet 5. The highest mean percentage of adult females (55%) occurred when the larval stages fed in on diets, 3 & 4 whereas the lowest one (45%) was obtained in the case of larval feeding on artificial diet, 5.

### 3.2.3. Female fecundity

With regarding to Table 2, data was revealed to a significant difference between the total deposited number of eggs by a single mated female after the rearing of larvae on the five tested diets. The total number deposited of eggs/female could be arranged in descending order as 254.00 ± 6.78 204.00 ± 6.00, 218.00 ± 8.00, 202.00 ± 5.83 and 198.00 ± 7.35 eggs/ ♀ resulted after feeding the larval stages on 4<sup>th</sup>, 5<sup>th</sup>, 2<sup>nd</sup>, 1<sup>st</sup> and 3<sup>th</sup> diets, respectively. A significant difference was observed between the female fecundity with fourth artificial diet and other tested diets (Table 2).. The eggs that were obtained after rearing the larvae on each of the tested diets were kept until hatching. The highest mean of hatchability % (96.53 ± 0.48%) occurred when larvae fed on 4<sup>th</sup> diet, which it was significantly higher the other four diets. The lowest hatching % (95.41 ± 0.17%) was obtained in case of 2<sup>nd</sup> diet i.e. sweet pumpkin diet.

### 3.2.4. Effect of the diet type on the measurements of the resultant adults

Data presented in Table 3 showed that the dimensions (length and width) of the RPW adult were significantly affected by the food type variations in the larval development. The biggest adult of RPW (3.44 ± 0.04 cm long and 1.30 ± 0.00 cm wide) were obtained when larvae were fed on 4<sup>th</sup> diet, whereas, the shortest adults' length (3.34 ± 0.02 cm) and width (1.12 ± 0.02 cm) were found with 1<sup>st</sup> diet.

### 3.2.5. Weight of larva and adult

The weight of RPW larvae and adults were significantly influenced by variations in the kind of larval food. The heaviest weight of both larvae and adults (6.70 ± 0.15 and 1.41 ± 0.01) obtained with 4<sup>th</sup> diet, whereas the lightest adults' weight (1.32 ± 0.01 g.) was obtained in case of 3<sup>rd</sup> diet (Table 4).

The success of the five tested diets in providing the necessary nutritional requirement for development and molting of the red palm weevil was based on the data recorded on the different life parameters of the *R. ferrugineus* reared on these diets. The life parameters included eggs' productivity/female, egg viability (hatching %), developmental periods of immature stages, fecundity and female: male ratio. The superior diet was 4<sup>th</sup> diet (sweet potato), this may be attributed to the better data on biology, and the diet retained moisture and remained fresh all the time.

### 3.3. Biochemical components in *R. ferrugineus* larvae after the feeding on different diets

With regarding data presented in Table 5, it revealed that the highest amount of total protein in RPW full-grown larvae (28.46 ± 0.55, µg/g) was detected when the rearing was taking place on 4<sup>th</sup> diet (sweet potato diet; 28.46 µg/g) being, significantly, higher than that recorded in case of the rearing on 3<sup>th</sup> diet (ground sugarcane + sweet pumpkin) which caused the lowest amount of protein content (25.99 ± 0.22 µg/g). The obtained results showed that a detectable increase in the total carbohydrates (45.57 ± 2.92 µg/g) when the rearing was taking place on 1<sup>th</sup> diet (sugarcane stem pieces). Contrariwise, a significant decrease in total carbohydrates (39.48 ± 0.57 µg/g) occurred when the larvae were reared on 3<sup>rd</sup> diet (Table 5). The total lipid content

**Table 3.** Morphometric measurements of *Rhynchophorus ferrugineus* adults in the laboratory after reared on different diets (Mean ± SE).

Measurements	Artificial Diets				
	1 <sup>st</sup> Diet	2 <sup>nd</sup> Diet	3 <sup>rd</sup> Diet	4 <sup>th</sup> Diet	5 <sup>th</sup> Diet
L (cm)	3.34 ± 0.02 <sup>ac</sup>	3.42 ± 0.04 <sup>aAB</sup>	3.36 ± 0.02 <sup>aBC</sup>	3.44 ± 0.04 <sup>aA</sup>	3.36 ± 0.02 <sup>aBC</sup>
W (cm)	1.12 ± 0.02 <sup>bb</sup>	1.16 ± 0.02 <sup>bb</sup>	1.16 ± 0.04 <sup>bb</sup>	1.30 ± 0.00 <sup>ba</sup>	1.26 ± 0.02 <sup>ba</sup>
LSD at 0.05	L+W = 0.04		Diet = 0.06	L*D = 0.08	

Values denoted by the same lowercase letter in the same column are non-significantly different, while, it signed by the same uppercase letter in the same row are non-significantly different at P>0.05. L= Length ; W= Width.

**Table 4.** Effect of the feeding on different diets on the weight of larvae and adults (Mean ± SE).

Stage	Artificial Diets				
	1 <sup>st</sup> Diet	2 <sup>nd</sup> Diet	3 <sup>rd</sup> Diet	4 <sup>th</sup> Diet	5 <sup>th</sup> Diet
Larvae (g.)	5.90 ± 0.18 <sup>cd</sup>	6.20 ± 0.25 <sup>abc</sup>	6.10 ± 0.18 <sup>ac</sup>	6.70 ± 0.15 <sup>aA</sup>	6.30 ± 0.21 <sup>aB</sup>
Adult (g.)	1.35 ± 0.02 <sup>ba</sup>	1.37 ± 0.02 <sup>ba</sup>	1.32 ± 0.01 <sup>ba</sup>	1.41 ± 0.01 <sup>ba</sup>	1.36 ± 0.02 <sup>ba</sup>
LSD at 0.05	Diet = 0.28		Stage = 0.18		D*S = 0.39

Values denoted by the same lowercase letter in the same column are non-significantly different, while, it signed by the same uppercase letter in the same row are non-significantly different at P>0.05.

**Table 5.** Impact of the rearing on artificial diets on the biochemical components in *Rhynchophorus ferrugineus* (Mean  $\pm$  SE).

Diets	Components ( $\mu\text{g/g}$ )		
	Total protein	Total carbohydrates	Total lipids
1 <sup>st</sup> Diet	27.63 $\pm$ 0.91 <sup>ab</sup>	45.57 $\pm$ 2.92 <sup>a</sup>	18.16 $\pm$ 0.58 <sup>ab</sup>
2 <sup>nd</sup> Diet	27.07 $\pm$ 0.64 <sup>ab</sup>	41.91 $\pm$ 1.03 <sup>ab</sup>	18.41 $\pm$ 0.94 <sup>ab</sup>
3 <sup>rd</sup> Diet	25.99 $\pm$ 0.22 <sup>b</sup>	39.48 $\pm$ 0.57 <sup>b</sup>	17.39 $\pm$ 0.48 <sup>a</sup>
4 <sup>th</sup> Diet	28.46 $\pm$ 0.55 <sup>a</sup>	42.81 $\pm$ 1.23 <sup>ab</sup>	20.01 $\pm$ 0.94 <sup>b</sup>
5 <sup>th</sup> Diet	27.79 $\pm$ 0.29 <sup>ab</sup>	42.81 $\pm$ 1.23 <sup>ab</sup>	19.01 $\pm$ 0.94 <sup>a</sup> <sup>b</sup>
LSD at 0.05	1.83	5.06	2.53

Values signed by the same lowercase letter in the same column are non-significantly different at  $P > 0.05$ .

could be arranged from 17.39  $\pm$  0.48 (Diet, 3) and 20.01  $\pm$  0.94  $\mu\text{g/g}$  (Diet, 4). Thus, confirming the highest amount of total lipids by rearing on 4<sup>th</sup> diet (sweet potato diet).

#### 4. Discussion

Additional studies are required to search for alternative food sources to develop new diets to substitute sugarcane stem pieces (the natural diets) for easier laboratory rearing, so that *R. ferrugineus* can fully develop on it. The larval development of the red palm weevil, *R. ferrugineus*, proved to be influenced by feeding on different diets. The five artificial diets used succeeded in providing the necessary nutritional requirements for the red palm weevil's growth and development. The appropriateness of the artificial diets can be measured by comparing their impact on various biological parameters including: 1) fecundity or the number of deposited eggs per female, 2) eggs' viability or percentages of fertile eggs hatching, 3) larval development time, 4) larval biomass, 5) pupal period, 6) larval survival percentage, 7) adults' emergence and 8) female: male ratio, as well as mentioned by Mahmoud et al. (2015), Abdel-Hameid (2019), El-Zoghby and Abdel-Hameid (2018). The obtained data (Tables, 1-5) confirmed that 4<sup>th</sup> diet (basically dependent on sweet potato) could be considered the best for rearing *R. ferrugineus*, it may be due to the sweet potato contained a good amount of protein, fat, energy, carbohydrate, ash, crude fibre,  $\beta$ -carotene and vitamin-C and also it contains better amount of minerals, potassium, magnesium, zinc, iron, copper, manganese and sodium (Senthilkumar et al., 2020).

The artificial diet, 4 (composed from sweet potato, agar, dried active yeast, sorbic acid, L- ascorbic acid, sodium benzoate, and distilled water) appeared to enhance the growth rate better than other diets, resulting in heavier larvae that completed their development in a shorter period of time. This diet, due to its ingredients, made a good feeding mixture that supported larval growth and metamorphosis. Higher protein food sources primarily work as an insect body builder, protein affects larval development by synthesizing body tissues and hormones (Fagbohun et al., 2012). Additionally, Salama and Abdul Razeq (2002) stated that this insect species was successfully reared on diets having high sugar contents as those

obtained in this study in which basically dependent on sweet potato. When feeding the red palm weevil on sweet potato (diet 4), the highest mean of total numbers of eggs/♀ (254 eggs/female) was recorded. In this respect, Hussein (1998) reported that female throughout its whole life, lays from 200 to 400 eggs. However, Salama et al. (2009) reported that the egg production rate was the lowest (117  $\pm$  18.9 eggs/♀) from rearing on sugarcane.

Data of the present study showed that the larvae fed on sugarcane stem pieces exhibited the longest duration of 144 days; these results agreed with Mahmoud et al. (2015), they studied the effect of diets on some biological aspects to the red palm weevil under laboratory conditions (sugar cane slices, semi-artificial diet and palm heart). The larvae fed on sugar cane slices slowly developed to exhibit the longest duration of 179.19 days. Several attempts have been made by researchers to rear red palm weevils on non-host plants including sweet potato tubers. El-Mergawy and Al-Ajlan (2011) and El-Sebay (2003) created a semi-synthetic diet made from potatoes or sweet potatoes, carrot, glucose, casein, agar, cereals, vitamin B, vitamin D, and water. The weevils reared on these diets successfully completed their life cycle and produced new generations.

#### 5. Conclusion

This study demonstrated that the life-cycle parameters of RPW are affected by the type of diets used. A sweet potato diet gave the best results for larval growth, metamorphosis, and female fecundity. Therefore, this diet (Diet, 4) could be recommended as a suitable and economical artificial diet for the laboratory rearing of *R. ferrugineus*. This study may help to improve each component of the RPW-IPM program based on a much better knowledge on suitable artificial diet of this pest.

#### Acknowledgements

The author wishes to express her deep and sincere thanks to Dr. Fawzy Faiek Shalaby, Emeritus Professor of Economic Entomology, Plant Protection Department, Faculty of Agriculture, Benha University, for suggesting



the problem, keen and close help, scientific guidance, valuable and fruitful advices and constructive criticism throughout the whole period of this study and also for revising the manuscript.

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