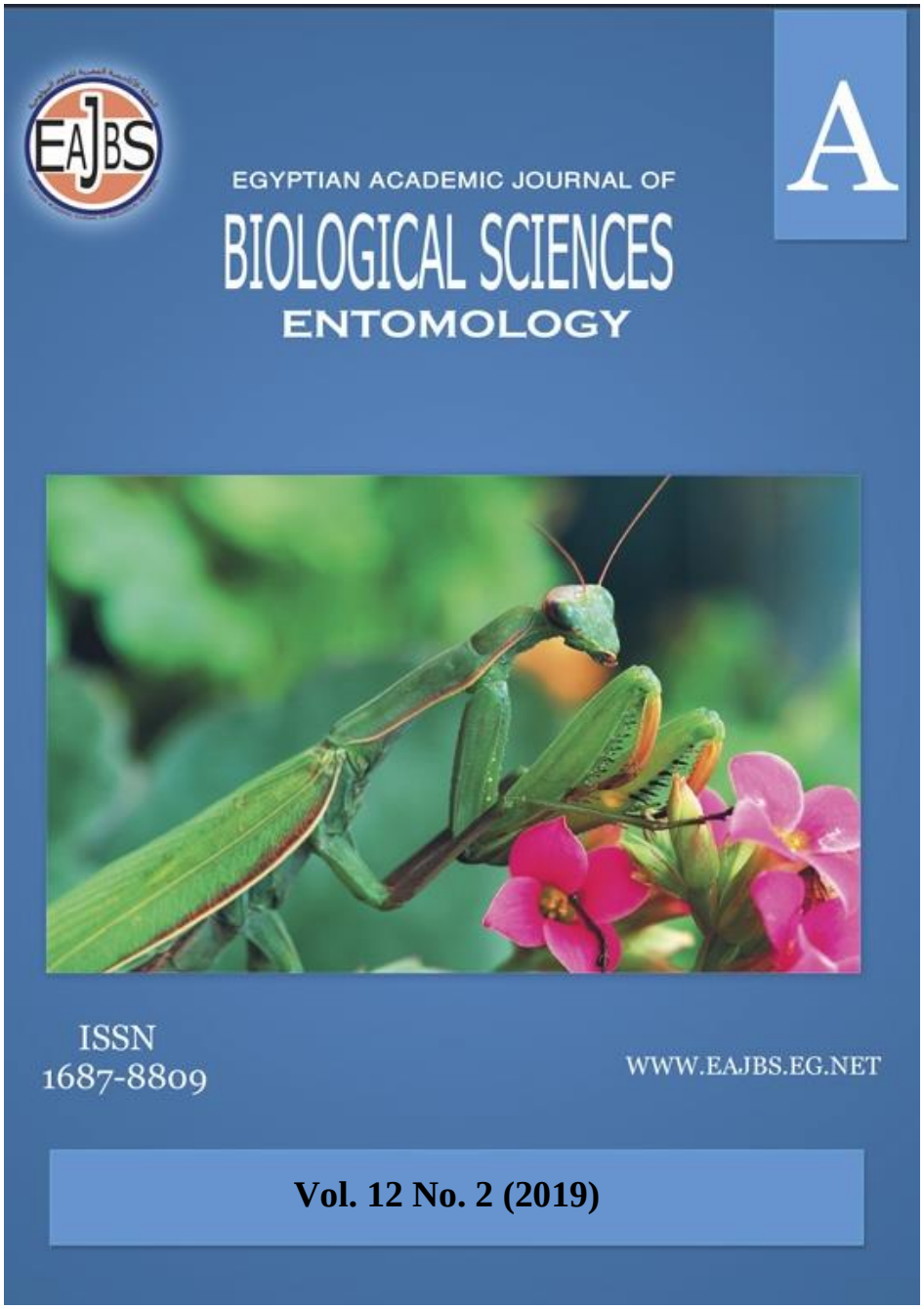

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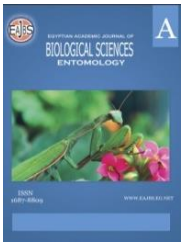

Feeding of the Red Palm Weevil, *Rynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) Larvae on Natural and Artificial Diets in Relation to Biology and Chemical Contents.

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ABSTRACT

Life parameters including pre-oviposition, oviposition and larval & pupal periods, adult male and female longevities ,and generation period were recorded for the red palm weevil, *Rhynchophorus ferrugineus* Olivier reared on the sugarcane stem pieces (Diet,1) and other 3 artificial diets depending mainly on ground sugarcane ,(diet 2); ground corn , (diet 3) and ground mixture of sugarcane and corn (diet 4) . In the laboratory at 28±1°C and 75±5% R.H. , the effects of all diets on total proteins , carbohydrates and lipids were also determined in the last instar larvae .Larvae fed on diet 2 (ground sugarcane + additives) exhibited shortest larval duration (86.30 days) , while diet 3 (ground corn + additives) caused the longest larval period (128.35 days) . Shortest and longest pupal periods resulted also from feeding on diet 2 and diet 3 (15.3 and 21.75 days , respectively) . On the other hand , male and female longevities were the longest and shortest when previous larvae were fed on diet 2 and diet 3 (50.4 and 38.4 days for males and 48.0 and 36.8 days for females , respectively . The sex – ratio was almost 1:1 with all diets , except diet 2 which led to more females (1 male : 1.5 female).Eggs of *R. ferrugineus* hatched after about 4 days , but this period was , significantly , shorter (3.35 days) by rearing on diet 2 . Highest hatch ability percentage (93.61 %) resulted from rearing on the same diet .

Chemical analyses of last larval instar content indicated that the highest total contents of proteins (51.87 mg / g) , carbohydrates (105.0 mg / g) and lipids (21.13 mg / g) were obtained by rearing on diet2, diet 1 and the control (natural feeding on palm), respectively .

INTRODUCTION

Date palm is the most common and widely cultivated plant in the arid regions of the Middle East and North Africa. Date fruits provide the staple carbohydrate food of people for nearly 5000 years (Purseglove, 1972 and Jones, 1995) .The date palm crop in these countries is nowadays under threat for the direct attack by the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera : Curculionidae) which is , fairly , considered one of the most invasive and destructive palm tree pests worldwide (Faleiro, 2006). Larvae feed inside the trunk and frequently destroy the apical growth area, causing the death of the palm trees (Murphy and Briscoe, 1999). Signs of infection are usually detected after the palm tree has been seriously damaged and only well-trained technicians can detect early symptomatology, so that numbers of plants are lost and the consequent

financial impact becomes considerable. The pest has a broad range of hosts and is able to breed in a wide variety of climatic conditions (Murphy and Briscoe, 1999).

Adult females of RPW lay eggs in protected parts of the date palm tree, including wounds on the trunk of trees, at the base of fronds, the crown of the tree and adjacent to offshoots. Early stages of attack are difficult to be detected, but it and can be cured with insecticides (stem injection). In later stages of the attack, palms harbor several overlapping life stages of the pest with extensive damage to tissues due to feeding by grubs, and so , those have to be eradicated (Faleiro, 2006).

Ideal diet for insect mass rearing programs should supply all nutrients needed for larval feeding, and should be easy to prepare and store for a long period . Also, it has to be inexpensive and should produce an average yield of acceptable adults. Many investigators used various feeding substrates for RPW rearing in the laboratory for experimental purposes. In many cases, the laboratory rearing and mass production of RPW is very important to maintain purity, age, physiological stage homogeneity and sex-based selection to carry out laboratory, semi-field and field experiments. The ability to produce quality-controlled insects in large numbers is essential for many research projects .The present study was carried out for rearing RPW on artificial diets hoping to be as an alternative to rearing on date palm tissues.

MATERIALS AND METHODS

Insects :

Samples of male and female adults of the red palm weevil (*R. ferrugineus*) were collected from date palm groves at Qassasin, Ismailia Governorate during 2018. The collected adults were used as the initial samples for rearing. The rearing process was carried out in a rearing room at means of 28±1°C and 75±5% R.H.

Diets Used for Rearing *R. ferrugineus* :

1-Duration of immature stages:

a-Egg Incubation Period :

1-Diet (1) Contains Small Pieces of Sugarcane Stem Pieces:

Freshly emerged *R. ferrugineus* adults obtained after rearing on different diets (20 pairs of newly emerged male and female weevils) were collected and reared in the containers with different diets. The insects were left for one week to ensure copulations. Deposited eggs were collected daily by peeling the fibrous tissues, picking up eggs using a camel's hair brush. Eggs were gently placed on wet filter papers to avoid dryness inside Petri - dishes (10 cm diameter) for the needed studies, where eggs were monitored until hatching.

b – Larval and Pupal Stages:

The freshly emerged larvae were , gently , removed and distributed in glass bottles (5 × 10 cm. ; Fig. 1) containing different diets . The bottles and larvae were, daily, observed until reaching the last larvae instar.

At this stage , the full – grown larvae and diets were translocated to plastic boxes with light pored covers (25×20×10 cm.; Fig. 2) , where pieces of sugarcane or corn stems were added to suitable media to help the larvae for pupation . The total larval period of larvae reared on different diets were recorded. The formed pupae were placed, singly, in small cups (5 ×5cm.) covered with pored lids and cups were observed in the laboratory unit weevils' emergence .

Adult's Stage:

The emerged adults were kept, every pair, in a cup (5 × 10 cm.) .containing small sugarcane stem pieces. The cups containing adults were daily observed and the sugarcane

pieces were removed, kept in other cups and replaced with new sugarcane pieces whenever needed. Adults were left in the cups until mortality when the male and female longevities were estimated and recorded.

Components and figures of the artificial diets used in the rearing of *R. ferrugineus*are as follows:

Component	Amount
Ground sugarcane	250 g
Agar	10 g
Dried active yeast	15 g
Sorbic acid	1.25 g
L- Ascorbic acid	2.50 g
Sodium benzoate	1.25 g
Distilled water	150 - 200 ml

A : Artificial diet 2

B : Artificial diet2 +Larvae

C:Artificial diet2+Last larval instars + Small pieces of sugarcane

(A)

Component	Amount
Ground Corn	250 g
Agar	10 g
Dried active yeast	15 g
Sorbic acid	1.25 g
L- Ascorbic acid	2.50 g
Sodium benzoate	1.25 g
Distilled water	200 - 300 ml

A: Artificial diet 3

B: Artificial diet3 +Larvae

C: Artificial diet3 + Last larval instars + Corn stem pieces

(B)

Component	Amount
Ground Sugarcane	125 g
Ground Corn	125 g
Agar	10 g
Dried active yeast	15 g
Sorbic acid	1.25 g
L- Ascorbic acid	2.50 g
Sodium benzoate	1.25 g
Distilled water	150 - 200 ml

A : Artificial diet4 +Larvae

(C)

Three artificial *R. ferrugineus* diets used for rearing larvae: A . Diet, 2. B . Diet, 3. C . Diet, 4.

Eggs' Productivity / Female:

The small sugarcane stem pieces containing eggs were dissected and deposited eggs were collected, counted and reared until hatching as mentioned by El-Zoghby and Naglaa (2018).

Fig.1: Artificial diet + Newly hatched larvae

Fig.2: Artificial diet + Last larval instars

Biochemical Assays:

Chemicals Used:

Bovine albumin standard was purchased from Stanbio laboratory (Texas, USA). Commassie brilliant blue G-250 was from Sigma (Sigma chemicals co.).P- nitroanisole (purity 97%)was obtained from Ubichem Ltd.(Hampshire),while reduced from of nicotinamideademine dinucleotide phosphate (NADPH)was from BDH chemicals Ltd.(Poole , England). The rest of the chemicals were of high quality and purchased from commercial local companies.

Apparatus:

Insects were homogenized in a chilled glass Teflon tissue homogenizer (ST – 2 Mechanic - Preczyina, Poland). After homogenization, supernatants were kept in a deep freezer at -20 °C until used for biochemical assays. Double beam ultraviolet / visible spectrophotometer (spectronic 1201, Milton Roy Co., USA) was used to measure the absorbance of colored substances or metabolic compounds.

Preparation of Full – Grown Larvae for Analysis:

The larvae were prepared as described by Amin (1998). Those were homogenized in distilled water (50 mg /1 ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min. at 2 °C in a refrigerated centrifuge. The deposits were discarded and the supernatants, which are referred to as enzyme extract, could be stored for at least one week without appreciable loss of activity at 50 °C.

Biochemical Assayed:

1-Determination of Total Lipids:

Total lipids were estimated according to the method of Knight *et al*. (1972) using phosphor vanillin reagent.

2-Determination of Total Carbohydrates:

Total carbohydrates were estimated in the acid extract of the sample by the phenol-sulphuric acid reaction of Dubies *et al*. (1956). Total carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967).

3-Total Proteins:

Total proteins were determined according to the method of Bradford (1976).

Statistical Analysis:

All data were analysed by using ANOVA with three factors at 0.05 significance level for the whole results using SPSS (ver. 22). Data were treated as complete randomization design according to Steel *et al*. (1997). Multiple comparisons of significance were carried out by applying LSD values.

RESULTS AND DISCUSSION

As for the total protein content, data presented in Table (1) indicated that the highest rate of total protein content in full – grown larvae (51.87±0.70 , mg/g) was detected when rearing took place on diet (2) (ground sugarcane + additives). That was non-significantly, followed by rearing on sugarcane stem pieces (48.97±0.48) and significantly by rearing on diet (4).

As for carbohydrate content, it showed detectable increase as it reached (105.00±4.04) , (84.50±1.61) and (70.00±1.15) when rearing took place on diet (1), diet (2) and diet (4) respectively , while diet (3) showed a significant decrease in total carbohydrate (55.53±1.07) , compared to control (64.77±1.94) .

As for lipid content , it showed a detectable decrease in total lipase, being (19.03±0.43), (18.37±0.37), (15.30±0.35) and (17.17±0.17) by rearing on diet (1), diet (2) , diet (3) and diet (4) respectively, compared to control (21.13±0.58 mg/g)

Table 1: Effect of different diets on the amount of biochemical components (mg/g)

	Diets	Total proteins	Total carbohydrates	Total lipids
1	The palm (Control)	43.33±1.42^{b}	64.77±1.94^{c}	21.13±0.58^{a}
2	Diet,1 (Sugarcane stems pieces)	48.97±0.48^{a}	105.00±4.04^{a}	19.03±0.43^{b}
3	Diet 2	51.87±0.70^{a}	84.50±1.61^{b}	18.37±0.37^{bc}
4	Diet 3	42.50±1.04^{b}	55.53±1.07^{d}	15.30±0.35^{d}
5	Diet 4	45.40±0.67^{b}	70.00±1.15^{c}	17.17±0.17^{c}

Values having the same superscript in the same column are no significant different.

Incubation Period Of Eggs:

Eggs' hatching occurred after 3.35±0.11 to 4.60±0.11days after rearing on artificial diet (2) & diet (3), respectively (Table, 2). Eggs obtained after rearing on diet (2) had , significantly , the shortest incubation period than the remaining diets .

Effect of Different Diets on the Total Larval Duration:

Data tabulated in Table (2) show the total larval period during the first generation of red palm weevil, *R. ferrugineus* fed on the succeeded different diets. Statistical analysis of data revealed significant differences in this period between different diets.

In similar studies, Kaakeh*et al.* (2001) reported that red palm weevil completed its larval period in 50-80 days .Salama & Abdul Razek (2002) stated that this insect species was successfully reared on diets having high sugar contents.

Table 2. Effect of different diets on some biological aspects of *R. ferrugineus* 28±1°C and 75±5% R.H

	Diets	Development time (mean, days)					
		Egg incubation period	Larva	Pupa	Total	Longevity	
						Male	Female
1	Diet,1 (Sugarcane stems pieces)	4.30±0.11^{a}	110.75±0.16^{c}	20.00±0.49^{b}	130.75±0.47^{c}	45.2±1.07^{b}	44.4±0.93^{b}
2	Diet 2	3.35±0.11^{b}	86.30±2.15^{d}	15.30±0.35^{c}	101.65±2.10^{d}	50.4±0.51^{a}	48.0±0.45^{a}
3	Diet 3	4.60±0.11^{a}	128.35±0.82^{a}	21.75±0.27^{a}	150.1±0.80^{a}	38.4±0.51^{c}	36.8±0.58^{d}
4	Diet 4	4.40±0.11^{a}	118.60±0.58^{b}	18.95±0.41^{b}	127.55±0.78^{b}	40.0±0.45^{c}	39.4±0.24^{c}

a, b & c: There is no significant difference (P>0.05) between any two means having the same superscript letter within the same column.

Duration of the pupal stage:

Statistical analysis of results showed significant differences between the pupal periods of pupae obtained after rearing on the tested diets. As shown in Table (2), the pupal period ranged from a minimum of 15.30±0.35 days when rearing took place on diet (2) to a maximum of 21.75±0.27 days by rearing on diet (3). During this period, the pupae remain in their cocoons until hatching of adults' (Fig. 3).

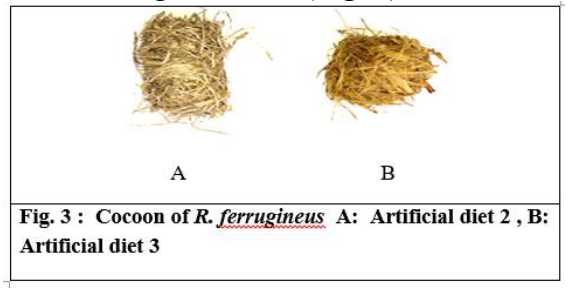

Fig. 3 : Cocoon of *R. ferrugineus* A: Artificial diet 2 , B: Artificial diet 3

Sugarcane stem pieces were suitable for improving pupation because the full-grown larvae used the fibers of these pieces to construct their cocoons , while, larvae failed to construct their cocoons , in any other diet (Kakeeh *et al*., 2001). The diet consisting of sugarcane stem pieces + food residues of RPW diet was found to be the best medium for the development of all larval stages to the pupal stage . El-Zoghby and Naglaa (2018) found that the pupal period ranged from a minimum of 21 days when rearing of larvae took place on small pieces of sugarcane stems to a maximum of 34 days by rearing on sugarcane stem pieces + ground of date palm frond.

Longevity of Adults:

Data presented in Table (2), showed that the rearing of previous larvae on the different tested diets had influenced this period. The type of larval diet proved to has, statistically, significant effect on the longevity of males and females. The longest means of longevity (50.4±0.51 days for males and 48.0±0.45 days for females) were obtained with adults emerged from larvae fed on the diet (2).

Sex - Ratio:

Data tabulated in Table (3), show the sex ratio among emerged adults (as percentage of emerged of females). The highest mean percentage of adult females (60 %) occurred between adults fed in the larval stage on diet (2), whereas the lowest one (45 %) was obtained in case of larval feeding on artificial diet 3.

Female Fecundity:

Statistical analysis of results given in Table (3) exhibited significant differences between the total number of eggs deposited by a single mated female resulted after rearing of larvae on the four tested diets . The obtained total number of eggs / female can be arranged descend as follows: 254.0±6.78, 180.4±10.23 , 162.0±5.83 and 146.0±5.10eggs/female resulted by feeding of larvae on ; diet (2) , diet (1) , diet (4), and diet (3), respectively).Eggs obtained after rearing on each of the tested diets were kept until hatching . The highest mean percentage of hatchability (93.61±1.08 %) occurred when larvae were fed on diet (2) being, significantly, higher than those obtained from the three other treatments. Whereas the lowest one (78.01±1.38 %) was obtained in the case of diet (3). Results obtained by Kaakeh *et al*. (2001) indicated no significant difference in the percentage of hatchability of RPW eggs in cotton wool having a honey solution and Petri-dish lined with moistened filter paper as compared to other diets. El-Zoghby and Naglaa (2018) found that the highest mean percentage of hatchability (86.01%) occurred with larvae fed on (sugarcane stem pieces + food residues of RPW diet), whereas the lowest one (83.62%) was obtained in case of (sugarcane stem pieces + ground frond of date palm diet).

Table 3: Eggs productivity by *R. ferrugineus* females reared in their larval stage on different diets

	Diets	No. eggs/female	No.of Hatched eggs	% Hatching	Sex ratio %	
					Male	Female
1	Diet,1 (Sugarcane stems pieces)	180.4±10.23^{b}	152.2±7.50^{b}	84.51±0.81^{b}	50	50
2	Diet 2	254.0±6.78^{a}	238.0±8.60^{a}	93.61±1.08^{a}	40	60
3	Diet 3	146.0±5.10^{c}	114.0±5.10^{c}	78.01±1.38^{c}	55	45
4	Diet 4	162.0±5.83^{bc}	134.0±5.79^{bc}	82.67±1.56^{b}	50	50

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column having the same superscript letter.

Data presented in Table (4), show that the adult of RPW length and width was significantly affected by variations in the type of larval food. The biggest weevil adults (3.30±0.03 cm long and 1.20±0.00 cm wide occurred with larvae fed on diet (2) , whereas the smallest adult's length(3.04±0.02 cm.) and width (1.00±0.0 cm)were obtained in case of diet (3).

The success of the four tested diets in providing the necessary nutritional requirement for RPW development and molting was based on data recorded for various life parameters of *R. ferrugineus* reared on these diets. These life parameters included; eggs' productivity / female, egg viability (percentage of hatchability), developmental periods, pupal period of immature stages, the percentage of adults' emergence, fecundity and female: male ratio. The superior diet was diet,2 (ground sugarcane + additives) because of better data on biology and the diet kept moisture and remained fresh all the time.

Previous attempts that were made by several researchers showed that sugarcane was a good substitute of coconut for rearing *R. ferrugineus* (Rahalkar *et al*., 1972). (Rananavare *et al.* 1975) incorporated sugarcane in nutrient agar for the feeding young RPW larvae and sugarcane stem pieces for feeding of older larvae . Rahalkar *et al.* (1978 and 1985) improved the culture of *R. ferrugineus* by developing an artificial die composed of sugarcane bagasse (fiber), coconut cake, yeast, sucrose, minerals, vitamins, and preservatives.

Table 4: Morphometric of *R. ferrugineus*in adults emerged in the laboratory after rearing on different diets.

Diets		length /cm	Width / cm
1	Diet,1(Sugarcane stems pieces)	3.20 ± 0.03^{b}	1.12 ± 0.02^{a}
2	Diet 2	3.30 ± 0.03^{a}	1.20 ± 0.00^{a}
3	Diet 3	3.04 ± 0.02^{c}	1.00 ± 0.03^{b}
4	Diet 4	3.08 ± 0.02^{c}	1.02 ± 0.05^{b}

, b & c: There is no significant difference (P>0.05) between any two means having the same superscript small latter in the same column .

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ARABIC SUMMARY

تغذية يرقات سوسة النخيل الحمراء ريكوفوريس فيريجنيس على بيئات طبيعية و صناعية وتأثيرهاعلى ببيولوجية الحشرة ومحتوياتها الكيميائية

نجلاء فكرى عبد الحميد
قسم وقاية النبات ، كلية الزراعة ، جامعة بنها

تم دراسة الفترات الحياتية لسوسة النخيل الحمراء ، و *Rhynchophorus ferrugineus* Olivier بما في ذلك فتره ما قبل وضع البيض ، وفتره وضع البيض وفتره العمر اليرقي والعذارء ، وطول العمر عند الذكور والإناث والتي تم تربيتها على قطع جذع قصب السكر (بيئه 1) ومطحون قصب السكر مع الاضافات (بيئه 2) ؛ ذرة مطحونة مع الاضافات (بيئة 3) وخليط من قصب السكر والذرة المطحون مع الاضافات (بيئة 4) .

تمت الدراسة في المختبر عند درجه حرارة 28 ± 1 درجة مئوية ودرجة رطوبة 5±75٪، تم تحديد آثار جميع البيئات على المحتوي الكلي للبروتين والكربوهيدرات والدهون في الطور اليرقي الاخير.

سجلت اليرقات التي تغذت على النظام الغذائي 2 (قصب السكر المطحون + الإضافات) أقصر مدة للعمر اليرقي (86.30 يومًا) ، بينما تسببت الحمية 3 (مطحون الذرة + الإضافات) في أطول فترة للعمر اليرقي (128.35 يومًا). كما نتجت الفترات الأقصر والأطول من فترة طور العذراء عن التغذية على الحمية 2 والنظام الغذائي 3 (15.3 و 21.75 يومًا على التوالي). من ناحية أخرى ، كان طول عمر الذكور والإناث هو الأطول والأقصر عندما تم تغذية اليرقات السابقة على النظام الغذائي 2 والنظام الغذائي 3 (50.4 و 38.4 يومًا للذكور و 48.0 و 36.8 يومًا للإناث ، على التوالي. كانت النسبة الجنسية تقريبًا 1: 1 مع جميع البيئات ، باستثناء النظام الغذائي 2 الذي أدى إلى مزيد من الإناث (1 ذكر: 1.5 أنثى) .

كان فقس البيض لسوسة النخيل الحمراء بعد حوالي 4 أيام ، لكن هذه الفترة كانت أقصر (3.35 يومًا) عن طريق التربية على الحمية 2. كذلك سجلت أعلى نسبة للفقس (93.61٪) عند التربية على نفس الحمية.

أشارت التحاليل الكيميائية لمحتوى الطور اليرقي الأخير إلى أنه تم الحصول على أعلى المحتويات الكلية للبروتينات (51.87 ملغم / جم) والكربوهيدرات (105.0 ملغم / جم) والدهون (21.13 ملغم / جم) من خلال التربية على النظام الغذائي 2 والنظام الغذائي 1 والكنترول (التغذية الطبيعية على النخيل) ، على التوالي.