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MORPHOMETRICAL AND HISTOLOGICAL STUDIES OF DISSECTED FEMALE REPRODUCTIVE SYSTEM OF THE RED PALM WEEVIL, *Rhynchophorus ferrugineus* (OLIVIER) (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Using light microscopy, the ovarian structure and oogenesis in the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) were described. Reproductive system consists of two ovaries, lateral oviduct, common oviduct, the vagina, the bursa copulatrix and spermatheca. Ovarioles are of polytrophic type and each ovariole is long and divided into three regions: the germarium, the vitellarium (early previtellogenic zone and vitellogenic zone) and the stalk or calyx. Study describes the morphological differentiation in the structure of the ovarioles in different ages of female (4, 6, 10, 20, 30, 40, 50 and 60 days-old weevils). The mean length of previtellogenic zone showed the highest value (9.89 ± 0.77 mm) in 30 days-old weevils while the lowest value (4.22±1.65 mm) was recorded in 60 days-old weevils. Vitellogenic zone mean length reached its maximum value (32.17±0.43 mm) in 20 days-old weevils, then decreased until reached its minimum one (18.11±4.55 mm) in 60 days-old weevils. Calyx showed stable length (3.00 mm) from 4th day to 60th day, while the mean width increased gradually until the 60th day (1.82±0.03 mm). The highest mean number of oocytes in previtellogenic zone/ 2 ovaries (32.44±0.77 oocytes) was recorded in 20 days-old weevils. While the lowest mean number of oocytes (11.55±4.29 oocytes) was recorded in 60 days-old weevils. In vitellogenic zone, mean number of immature oocytes/ 2 ovaries reached its maximum values in 20 days-old weevils with values of 62.66±3.53 oocytes, then decreased to record its minimum value (20.44±5.55 oocytes) in 60 days-old weevils. The highest period of mature ova production (calyx/ 2 ovaries) was 30-40 days post weevils emergence with mean of 21.66± 4.71 and 10.11±1.07 ova, respectively, then the number of mature ova decreased in calyx as it recorded minimum number of ova (4.33 ± 0.33) at the age of 60 days. The total mean of mature ova and immature oocytes production inside the reproductive system reached its maximum (118.77 oocytes) at age of 30 days. This method could be of a useful help in determining the age of female captured in traps in the fields.

Key words: Red palm weevil, morphology, ovariole, oogenesis.

INTRODUCTION

The red palm weevil, *Rhynchophorous ferrugineus* (Olivier) (Coleoptera: Curculionidae), is an economically important, tissue-boring pest of date palm in many parts of the world including Saudi Arabia, United Arab Emirates, Sultanate of Oman, and Egypt (Cox, 1993; Kaakeh *at al.*, 2001). The insect was first described in India as a serious pest of coconut palm (Lefroy, 1906; Nirula, 1956) and later on date palm (Lal, 1917; Buxton, 1918). Determination of the age structure of adult insect populations can improve our understanding of the population dynamics of a species, particularly one with overlapping generations. Age structure can also be used to construct time-specific life-tables to characterize factors that regulate fluctuations in population size and dispersal rate, as well as to monitor fertility, mortality, simulation models developed to predict populational responses to control programs or to predict outbreaks of the pest in response to environmental changes (Tyndale-Biscoe, 1984). Methods for age

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determination of insects are based primarily on changes in the reproductive system, changes in cuticle structure, or somatic changes (Hayes and Wall, 1999). Changes in the ovarian system can measure physiological age of the insect. Age-grading techniques based on physiological changes in the reproductive system include physical changes in ovaries (Tyndale-Biscoe, 1984; Hayes and Wall, 1999). Therefore, the present study is an attempt to describe a method of age grading weevils that is based on growth changes in the reproductive system.

MATERIALS AND METHODS

Cocoons were collected from heavily infested date palm trees at Kassasin district, Ismailia Governorate, Egypt and kept in rearing room till adult emergence, then the insects were sexed.

Weevils were divided into three test groups (n=50/group, 30 females and 20 males just after emergence through one day old). In each group, three females were dissected at different time periods (4, 6, 10, 20, 30, 40, 50 and 60 days), (8 ages×3♀/group).

Abdomen of females was dissected on dissecting plate provided with saline solution (9.0g NaCl, 0.2g KCl, 0.2g $CaCl_2$ and 4.0g sucrose per 1 L). The reproductive system was photographed and morphology of each was conducted under dissecting microscope and described. Thereafter, the ovariole end was grasped and pulled gently across a thin layer of saline solution on a clean slide until the ovarioles were straightened. The length of the ovariole zones were measured using an ocular micrometer inserted in the microscope.

RESULTS AND DISCUSSION

Morphology of Female Reproductive System

The morphology of female reproductive tract of *R. ferrugineus* was described at the age between 4 days and 60 days old in order to determine age-grading criteria.

The internal reproductive organs of *R. ferrugineus* females consist of two ovaries, two lateral oviducts, common oviduct (as the follicle passes through the constricted opening of the lateral oviduct, the follicular epithelium is stripped from the follicle and the egg passes into the lateral oviduct then the lateral oviducts from the two ovaries unite to form the common oviduct that joins the bursa copulatrix (Fig. 1 B), the bursa copulatrix, the vagina and spermatheca (Fig. 1 C). Each laterally placed ovary has two polytrophic ovarioles. Each ovariole is long and divided into three regions: germarium zone which produced oogonia (seem as many divided nuclei spread in this zone (Fig. 1 E), the more distal and somewhat enlarged in the end to terminate with swollen part (Fig.1 D), vitellarium zone which divided to early previtellogenic zone in which the oocytes were transparent and vitellogenic zone in which the oocytes took the white colour as the yolk began to be formed so vitellogenic zone contains a series of follicles (oocytes with a surrounding follicular epithelium) in successive stages of development and the third is the stalk (calyx) with mature ova with maximum volume, so groups of oocytes based on their age and morphology in ovariole divided into three groups in three regions: previtellogenic zone, vitellogenic zone and the calyx. This descriptive division of the oocytes was similar to the description of Sheng-wei *et al.* (2007) while studying oogenesis in the rice water weevil females, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), as they divided the oocytes in summer weevils into three groups based on their age and morphology. The first group was in early previtellogenic to middle vitellogenic stages. The second group was in late vitellogenic stage, oocyte volume had not reached the maximum, and chorion formation had not been completed. The third group was mature eggs with maximum volume.While the terminal filament is absent (Fig.1 A). Snodgrass (1935) mentioned that in some insects the terminal filaments are not united with one another, and in a few cases they are absent. This description of reproductive system disagrees with that mentioned by Kamel *et al.* (2005) who reported that the bursa copulatrix is absent and the vagina is straight and narrow and sclerotised posteriorly; El-Naggar *et al.* (2010) and Al-Dhafar and Sharaby (2012) who reported that each ovariole ends in a terminal filament, which unites with the filament from the other ovariole to form a short ligament.

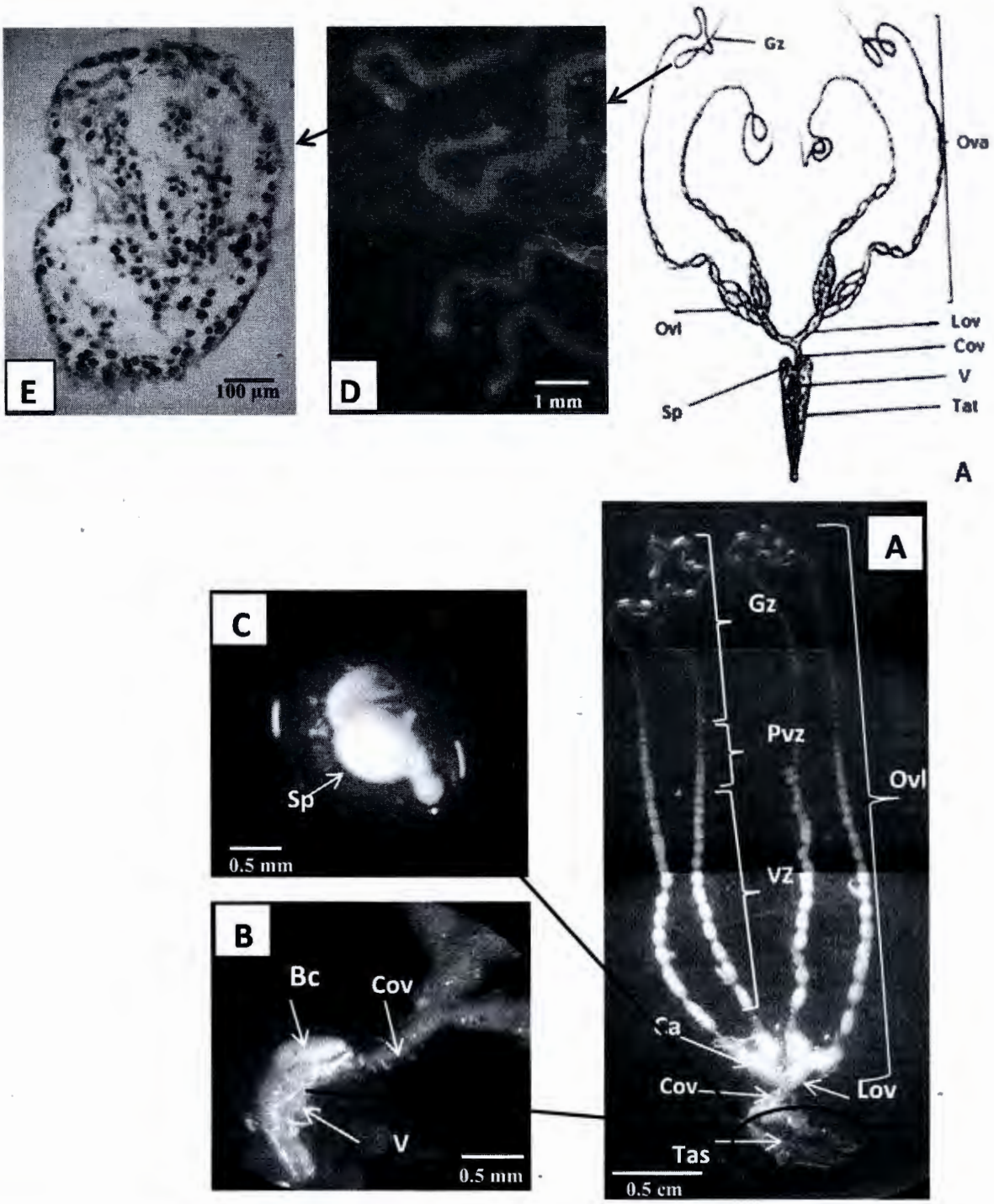

Fig. 1. A- Female reproductive system of the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.), showing germarium zone (Gz), vitellogenic zone (Vz), previtellogenic zone (Pvz), calyx (Ca), lateral oviduct (Lov), common oviduct (Cov), ovariole (Ovl), ovary (Ova), terminal abdominal sterna (Tas). B- Bursa copulatrix (Bc) and vagina (V). C- spermatheca (Sp). D- Terminal parts of the ovarioles germarium zone. E- Longitudial section in terminal part of germarium zone

Morphometric Measurements of Ovariole Zone Lengths (in mm)

Previtellogenic zone

Data presented in Table 1 demonstrate that the highest mean length of previtellogenic zone (9.89 ± 0.77 mm) was recorded in 30 days-old weevils, while the least value (4.22±1.65 mm) was recorded in 60 days-old weevils with general mean of 8.07±1.73 mm during weevils oviposition period.

Vitellogenic zone

Vitellogenic zone mean length started with (20.44 ± 0.40 mm) that increased gradually until reached its maximum value (32.17±0.43 mm) in 20 days old weevils, then decreased until reached its minimum value (18.11±4.55 mm) in 60 days-old weevils. The general mean of vitellogenic zone length during oviposition period was 26.62±5.27 mm. Fig. 2 showed that the length of the ovarioles was short at the beginning of oocytes formation at 4 and 6 days-old weevils, then increased in length at 10, 20 and 30 days-old weevils then shrinked as the ovarioles decreased in length and width and appear as short filaments at the end of females oviposition period.

Stalk or Calyx zone

Calyx showed stable length (3.00 mm) during the weevils oviposition period from the 4th day to 60th day, while the calyx mean width increased gradually from early oogenesis (1.20 ± 0.07 mm) with range of (1.13-1.26 mm) until 60th day (1.82±0.03 mm) with range of (1.80-1.86 mm) with general mean of 1.49±0.21 mm.

The Number of Ovarian Oocytes (Determination of Oogenesis and Ovarian Development)

Previtellogenic zones/2 ovaries

Results obtained in Table 2 indicated that the highest mean number of oocytes in previtellogenic zone (32.44±0.77 oocytes) was recorded in 20 days-old weevils. While the lowest mean number (11.55±4.29 oocytes) was recorded in 60 days-old weevils with total mean of 212.42 oocytes produced in this zone during weevils oviposition period and (26.55±7.00 oocytes) as a general mean.

Vitellogenic zones/2 ovaries

In vitellogenic zone, mean number of immature oocytes reached its maximum values in 20 days-old weevils with values of 62.66 ± 3.53 oocytes, then decreased to record its minimum value (20.44 ± 5.55 oocytes) in 60 days-old weevils. The total mean of oocytes of different ages in the female reproductive system was 387.53 oocytes and its mean was 48.44±14.73 oocytes. Fig. 2 confirmed this, as it showed that the highest numbers of oocytes appear through the ovarioles of 10, 20 and 30 days-old weevils, then decreased and distances appeared between oocytes follicles through the ovarioles of 50 days-old weevils until this zone seem to be stripped from oocytes in 60 days-old weevils as the weevils approach from post oviposition period.

Stalk or Calyx zones/2 ovaries

It is noticed that the highest period of mature ova production in female reproductive system (calyx) was 30-40 days post weevils emergence with mean of (21.66±4.71 and 10.11±1.07) ova respectively, which were ready to be laid. Then the number of mature ova decreased in calyx until calyx zones began to strip from ova as it recorded minimum number of ova (4.33± 0.33 ova) with range of (4.00-4.66) ova at the age of 60 days. The total mean of mature ova presented in calyx zones during weevil oviposition period was 76.19 ova with general mean of 9.52±5.28 ova. In Fig. 4 the calyx seems swollen in 10, 20 and 30 days-old weevils, as it contains the highest number of oocytes due to the highest production of oocytes during this period of the females oviposition period.

Lateral oviducts

The two lateral oviducts contained lower mean number of mature ova in early oogenesis (0.55±0.19 ovum) with range of (0.33-0.66 ovum), followed by the highest mean number of mature ova occurrence in lateral oviducts (2.89± 0.20 ova) with range of (2.66-3.00 ova) at the age of 30 days, then decreased until late oogenesis recording (1.33± 0.33 ova) with range of (1.00-1.66) ova with total mean of 14.98 ova and general mean of 1.87±0.75 ova during weevils oviposition period.

Table 1. Measurements of vitellarium and calyx zones of ovarioles in mm in different ages of dissected females of *Rhynchophorus ferrugineus* (Oliv.)

Dissected female ages (in days)	Zone (in mm)					
	Vitellarium length				*Calyx width	
	Previtellogenic		Vitellogenic			
	Range	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean±S.D.
4	6.80-9.60	8±1.44	20.06-20.86	20.44±0.40	1.13-1.26	1.20±0.07
6	8.80-10	9.38±0.60	27.53-27.66	27.62±0.08	1.26-1.33	1.28±0.04
10	7.33-9.07	8.47±0.99	31.33-32.33	31.77±0.51	1.33-1.40	1.35±0.04
20	8-9.86	8.91±0.93	31.86-32.66	32.17±0.43	1.33-1.46	1.40±0.07
30	9-10.33	9.89±0.77	30-31	30.44±0.51	1.53-1.66	1.60±0.07
40	7.46-8.33	7.93±0.44	28.13-28.86	28.55±0.38	1.50-1.64	1.58±0.07
50	7.53-8	7.73±0.24	23.33-24.66	23.88±0.69	1.66-1.73	1.68±0.04
60	2.33-5.33	4.22±1.65	15-23.33	18.11±4.55	1.80-1.86	1.82±0.03
General mean	4.22-9.89	8.07±1.73	18.11-32.17	26.62±5.27	1.20-1.82	1.49±0.21

*Calyx length was stable (3.00 mm) during the different ages, while the width was differed.

Table 2. Number of oocytes in vitellarium and calyx zones for four ovarioles and lateral oviducts in different ages of dissected females of *Rhynchophorus ferrugineus* (Oliv.)

Dissected female ages (in days)	No. of oocytes per zone								
	Vitellarium zone				Calyx		Lateral oviduct		Total mean
	Previtellogenic		Vitellogenic						
	Rang	(Mean±SD)	Rang	(Mean±SD)	Rang	(Mean±SD)	Rang	(Mean±SD)	
4	24.00-33.33	28.89±4.68	42.66-45.33	44.00±1.34	4.66-6.33	5.66± 0.88	0.33-0.66	0.55± 0.19	79.10
6	26.66-33.33	31.11±3.85	53.33-54.66	54.22±0.77	8.00-8.66	8.33± 0.33	1.00-1.66	1.44± 0.38	95.10
10	26.66-32.00	29.33±2.67	56.00-64.00	60.44±4.07	8.33-8.66	8.55± 0.19	1.66-2.33	2.00± 0.34	100.32
20	32.00-33.33	32.44±0.77	58.66-65.33	62.66±3.53	9.33-10.00	9.78± 0.39	2.33-2.66	2.44± 0.19	107.32
30	30.66-33.33	32.00±1.34	61.33-62.66	62.22±0.77	16.66-26.00	21.66± 4.71	2.66-3.00	2.89± 0.20	118.77
40	22.66-25.33	24.44±1.54	45.33-49.33	47.11±2.04	9.33-11.33	10.11±1.07	2.33-2.66	2.44± 0.19	84.10
50	21.33-24.00	22.66±1.34	36.00-37.33	36.44±0.77	5.66-10.33	7.77± 2.37	1.66-2.00	1.89± 0.20	68.76
60	6.66-14.66	11.55±4.29	16.00-26.66	20.44±5.55	4.00-4.66	4.33± 0.33	1.00-1.66	1.33± 0.33	37.65
General mean	11.55-32.44	26.55±7.00	20.44-62.66	48.44±14.73	4.33-21.66	9.52±5.28	0.55-2.89	1.87±0.75	
Total mean	212.42		387.53		76.19		14.98		

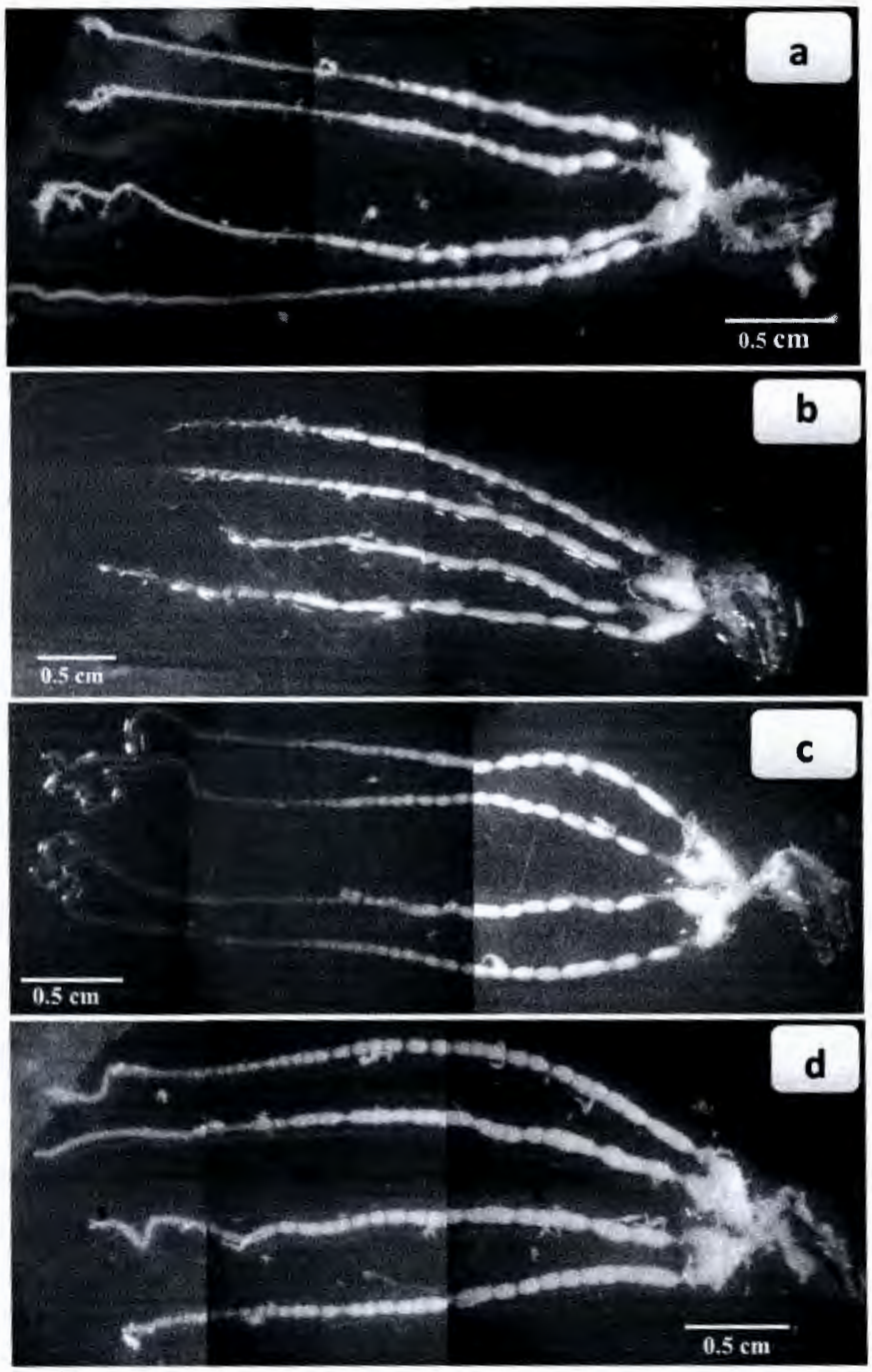

Fig. 2. Female reproductive system of the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) at a- 4 days-old weevil, b- 6 days old-weevil, c- 10 days-old weevil, and d- 20 days-old weevil

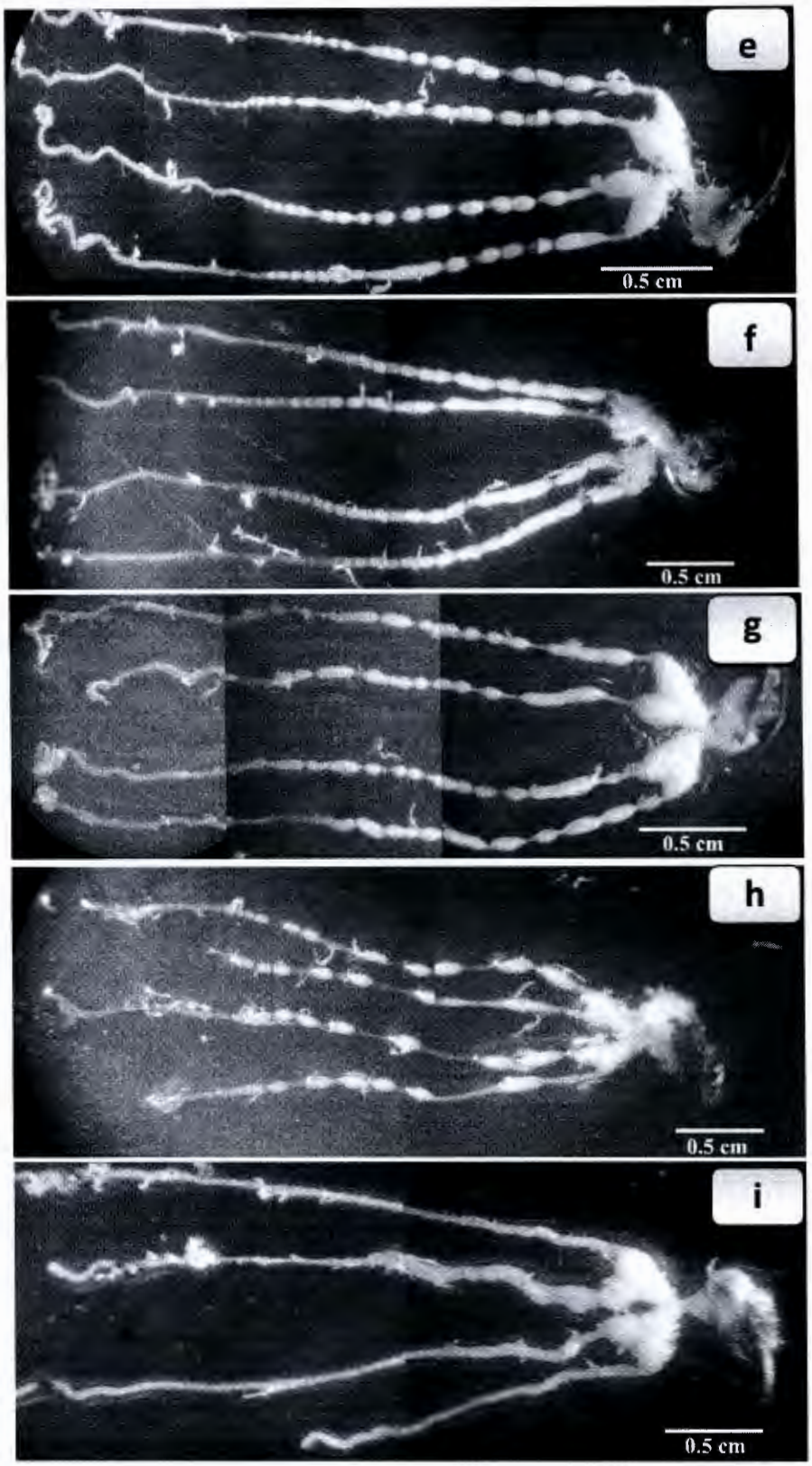

Fig. 2. Cont. Female reproductive system of the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) at e- 30 days-old weevil, f- 40 days-old weevil, g- 50 days-old weevil, h and i- 60 days-old weevil

The total mean of mature ova and immature oocytes production inside weevils ovaries (vitellarium zones, calyx zones and lateral oviducts) was 79.10 oocytes as a total mean at the age of 4 days, followed by increasing numbers until its maximum (118.77 oocytes) at the age of 30 days, then the oocytes number decreased until the ovary began to strip from oocytes at the age of 60 days recording 37.65 oocytes.

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دراســـات مـــورفـولـوجيـة علـى الجهـاز التناسـلى لإنـاث حشرة سوسة النخيل الحمراء

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تمت الدراسة المورفولوجية على الإناث لدراسة تركيب المبيض وتكوين البويضات فى حشرة سوسة النخيل الحمراء باستخدام الميكروسكوب الضوئى، واتضح من الدراسة ما يلي: يتكون الجهاز التناسلى من فرعين لكل مبيض وقناتى بيض جانبيتين وقناة بيض مشتركة ومهبل وكيس تلقيح وقابلة منوية، تعتبر فروع المبيض من النوع متعدد الخلايا المغذية ويتكون كل فرع من ثلاثة مناطق وهى المنطقة الجرثومية ومنطقة النمو (وتنقسم إلى منطقة ما قبل تكون المح ومنطقة تكون المح) والحامل (ويعرف بمنطقة الكأس ويحتوي على البيض الناضج)، تم تقدير المقاييس الخاصة بمنطقة النمو ومنطقة الكأس لفرع المبيض لإناث بأعمار مختلفة (٤، ٦، ١٠، ٢٠، ٣٠، ٤٠، ٥٠، ٦٠ يوماً)، حيث تظهر هذه الدراسة الوصف المورفولوجى لتركيب فروع المبيض، ولقد أوضحت أن منطقة ماقبل تكون أو ترسيب المح تحتوي على أعلى قيمة لمتوسط الطول فى إناث بعمر ٣٠ يوماً (٩,٨٩±٠,٧٧ مم) بينما كان أقل قيمة لمتوسط الطول فى إناث بعمر ٦٠ يوماً (٤,٢٢ ± ١,٦٥مم)، وصلت منطقة تكون أو ترسيب المح إلى أقصى متوسط للطول فى إناث بعمر ٢٠ يوماً (٣٢,١٧ ± ٠,٤٣ مم) ثم تناقص الطول إلى أقل قيمة فى إناث بعمر ٦٠ يوماً (١٨,١١ ± ٤,٥٥ مم)، أوضحت منطقة الكأس طول ثابت (٣,٠٠ مم) خلال فترة وضع البيض بينما تزايد عرض المنطقة تدريجيا حتى وصل لأقصى قيمة فى إناث بعمر ٦٠ يوماً (١,٨٢ ± ٠,٠٣ مم)، تم دراسة عدد البويضات فى منطقتى النمو والكأس لفرع المبيض وقناتى البيض الجانبيتين فى الأعمار المختلفة للإناث المشرحة حيث وصل عدد البويضات فى منطقة ماقبل تكون المح للمبيضين إلى أقصى عدد لها (٣٢,٤٤±٠,٧٧ بويضة) فى إناث عمر ٢٠ يوماً، بينما كان أقل قيمة له (١١,٥٥ ± ٤,٢٩ بويضة) فى إناث عمر ٦٠ يوماً، أما بالنسبة لمنطقة ترسيب المح وصل عدد البويضات للمبيضين إلى أقصى قيمة فى إناث عمر ٢٠ يوماً بقيمة (٦٢,٦٦ ± ٣,٥٣ بويضة) ثم تناقص ليصل إلى أقل قيمة فى إناث عمر ٦٠ يوماً بقيمة (٢٠,٤٤ ± ٥,٥٥ بويضة)، فى حالة منطقة الكأس كان أعلى فترة لإنتاج البيض الناضج (للمبيضين) فى إناث عمر ٣٠-٤٠ يوماً بمتوسط ٢١,٦٦ ± ٤,٧١ ، ١٠,١١ ± ١,٠٧ بيضة على التوالى ثم تناقص العدد ليسجل أقل قيمة (٤,٣٣ ± ٠,٣٣ بيضه) فى إناث بعمر ٦٠ يوماً، وصل المتوسط الكلى لإنتاج البيض الناضج وغير الناضج داخل الجهاز التناسلى لهذه الحشرة لأقصى قيمة (١١٨,٧٧ بويضة) فى إناث عمر ٣٠ يوماً، وهذه الطريقة سوف تفيد فى تقدير عمر الإناث التى يتم جمعها داخل المصائد فى الحقول.

المحكمون :

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