



RESEARCH PAPER

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Biological studies on palm tree weevil *Rhynchophorus Phoenicis fabricius* (Coleoptera; Curculionidae): An interest food bug in Côte d'Ivoire (West Africa)

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Abstract

Larvae of the palm tree weevil *Rhynchophorus phoenicis* are consumed and sold on the markets in Côte d'Ivoire, their high prices, in fact a luxury product. In order, to consider possibilities of domestication to increase their availability and decrease the cost, the objective of the present work is to know the development cycle of this species. To do this, a breeding was conducted at the laboratory of Zoology and Animal Biology of the University Felix-Houphouet-Boigny. The rearing device consisted of cylindrical and rectangular plastic boxes. The individuals used come from cocoons collected from palms infested in the field. As soon as the imagoes appeared, pieces of palm trunk were placed in these boxes to serve as support for spawning and nutrition. The total cycle time is 108.51 ± 3.6 days and has 6 larval stages, a nymphal stage followed by adult stage. Female lifetime fecundity was 252.26 ± 3.61 eggs. Incubation period was 4.09 ± 0.53 days with fertility rate of 88.30%. The mean larval and pupal development period were 33, 24 ± 0.9 days and 25.42 ± 2.4 days, respectively. The average emergence rate of adults was $88.30 \pm 2.04\%$. A significant difference was observed in adult life span ($F = 28.08$, $P < 0.05$). It is 68.86 ± 3.3 days in males and 54.71 ± 2.7 days in females. This work revealed the possibility of breeding *R. phoenicis* under controlled conditions. To avoid abusive harvests in already fragile ecosystem, breeding trials on other substrates would be possible.

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Introduction

The consumption of insects is a food practice that extends more and more in the world (FAO, 2013). Many African peoples consume large quantities even if the usually food tends to disappear gradually (van Huis *et al.*, 2013; Halloran *et al.*, 2014). The United Nations Food and Agriculture Organization (FAO) promotes since several years the use of insects in food and feed (FAO, 2010). Indeed, the consumption of insects expands, diversifies the diet, and helps prevent nutritional deficiencies (Malaisse, 2004). In West Africa, both termites, locusts, lepidopteran caterpillars and beetles are eaten. The larval and adult stages of *R. phoenicis* Fabricius (1801) commonly referred to as the caterpillar of the palm tree, are consumed in Côte d'Ivoire (Ouali and Ehounou, 2017). In addition, the commercialization of this species constitutes an important source of income for traders. In most cases, the insects consumed are directly obtained by harvesting or collecting in a natural environment. However, the availability is related to seasonal variations. The farms are still marginal and require a rigorous development to ensure a supply of quality and quantity for nutrition (FAO, 2013). In terms of rearing, insects have higher rates of growth and feed conversion rates and can breed on small spaces with a low impact on the environment (FAO, 2009; van-Huis, 2013). Irregular supply of markets in the larvae makes it difficult to meet demand especially during the dry season. To compensate these ruptures seasonal in supply, it is necessary to produce this insect outside of its natural habitat. The objective of this study is to know some biological parameters of *R. phoenicis*. Specifically, it will determine incubation period, female fecundity, egg fertility, the survival rate, larval development time, longevity, sex ratio and emergence rate of adults.

Materials and methods

Biological material

Biological material was *Rhyncophorus phoenicis*, Fabricius (1801) (Coleoptera; Curculionidae) commonly known as the caterpillar of the palm tree.

The individuals used in the adult stage, are derived from cocoons collected in a palm field at Ebimpé (-5.4946200° -4.0518300°) located in Anyama Côte d'Ivoire. They are fed by the palm tree (*Elaeis guineensis*) Jacq. of the Arecaceae family.

Collection and conditioning of cocoons

For the collection of the cocoons, the palm trees infested are identified through noises that make the larvae when they bore into the stipes. The palm is then opened using a machete. The cocoons, which is usually located at the peripheral of the stipe are harvested using a pair of pliers or by hand and stored in boxes in plastic with a lid perforated with holes of 19 cm x 6.5 cm x 28.2 cm (Fig. 1). These boxes are then transported to the laboratory to monitor the emergence of adults, which will be used for the study. Once at the laboratory, the cocoons were incubated individually in boxes plastic transparent perforated holes on the lid of dimension 18 cm x 12 cm (Fig. 2). The thermo hygrometers has been installed in the laboratory for the measurement of temperature and humidity. A reference number was assigned to each box containing the cocoons, to know the date of the harvest. The goal is obviously to determine the date of emergence of each adult. Upon emergence, adults were separated by sex based on external characteristics of the rostrum and then fed with the stipe of the palm (Fig. 2).

Study of the biological parameters

The biological parameters studied are: female fecundity, incubation period, eggs fertility; the duration of larval development, larval survival rate, the duration of the development cycle, adult longevity, emergence rate and the sex ratio. Experimental laboratory conditions were 27.86 ± 0.5 °C temperature and $74.15 \pm 2.2\%$ relative humidity (RH).

Pre-mating, pre-oviposition and fecundity

The experiments were initiated with thirty (30) pairs of *R. phoenicis* newly emerged represented by 30 males and 30 females. The date of emergence (J_e) of each individual was noted.

The couples were placed in boxes in plastic cylindrical dimension: 18 cm×12 cm containing pieces of stipe of the palm tree who have served in the faith of food to the couple and substrate of oviposition in the female. The boxes used have been numbered from 1 to 30 for the different experiments. Twenty-four (24) hours after spawning, each couple was transferred into a new box with the same characteristics as the first. The total number of egg deposited by female in the stipe is noted and the average fecundity (Fn) is calculated. The oviposition behavior is noted every day, until the death of the female.

Female fecundity (Fn) defines the average number of eggs laid by a female during its life and is expressed according to the following formula

$$\text{Female fecundity} = \frac{\sum \text{number of eggs laid}}{\sum \text{number of female}}$$

Eggs Fertility and incubation period

The eggs laid are incubated for 3 to 6 days in the box with transparent lid perforated with holes, useful for the renewal of the area and avoid molds. These boxes were monitored daily until the observation of the hatching of the eggs. After hatching, the larvae newly emerged are out of the boxes using flexible forceps and then the day of the outbreak (Dec) is noted to determine the average incubation period of the eggs. The average rate of fertility of eggs is the ratio, expressed as a percentage of the number of eggs hatched total number of eggs laid:

$$\text{Fertility rate} = \frac{\sum (\text{number of eggs hatched}) f_i}{\sum (\text{number of eggs laid}) f_i} \times 100$$

f_i: number of female

The average incubation period of eggs is the time between the date of spawning (Dp) and that of egg hatching (Dec).

$$\text{Incubation period (Di)} = \frac{\sum t_i v_j}{\sum v_i}$$

t_i = Dec – Dp ; *v_j* = number of egg hatched

Survival rate and larval development

After hatching, 300 larvae of stage 1 and aged one day were placed individually in boxes in transparent plastic (6x10x15 cm) perforated with holes of 5 mm diameter on the lids and then arranged on a wooden shelf.

The larvae were fed with pieces of a stipe of the palm tree of 25 g and 3 cm thick placed at the bottom of each box. The food was renewed every two or three days, when it seemed too dry or too moldy to the third stage. From the fourth to the last stage, the amount of food has been increased and it is increased to 35 g and 5 cm thick. The substrate was periodically washed down with palm wine in order to ensure a humidity favorable to the larval development. The boxes were monitored daily to record the date of molt for each larva by observing cast-off skins. A count of larvae from a lower stage to a higher stage has been made to determine the rate of larval survival (Si). The passage from one larval stage to another is marked by a molt, followed by the rejection of an exuvia. The size of this exuvia being greater than that of the previous stage. The average length of passage from one larval stage to the next was calculated and expressed in days. The measurements of the head capsule, the weight and the size of each stage were made using a vernier caliper and an electronic scale.

$$S_i = \frac{\sum (\text{number of larva of the next stage}) f_i}{\sum (\text{number of larvae from the previous stage}) f_i} * 100$$

f_i: number of females

Pupal development, rate of emergence and sex ratio

When the larva stage 6 completes its development, it begins to prepare for pupation. After the last molt, the larvae of stage 6 were placed in larger boxes (279 mm x 170 mm) containing, in addition to their support of nutrition, of palm fibers to allow for the construction of cocoons. The individuals were maintained in the same rearing conditions as those of the larvae of departure until the cocoon. Five cocoons were opened every 5 days to follow the evolution of the nymph to the adult. The dates of obtaining nymphs have been noted, and regular monitoring has been carried out, until the emergence of adults. The number and sex of the insects emerged were recorded each day until there is no more output. The rate of emergence (Te), which reflects the percentage of larvae stage I emerged (ni) on the average number of eggs laid (no) as well as the sex ratio reflecting the percentage of males emerged about one of the female masses were determined for the offspring of each female, according to the formulas:

$$Te (\%) = \frac{\sum nifi}{\sum nofi} \times 100$$

$$Sex - ratio (\%) = \frac{\sum numberofmales}{\sum numberoffemales} \times 100$$

Adults longevity and total duration of life cycle (DC) of R. phoenicis

The longevity of adults is reflected as the elapsed between the date of emergence (Je) of the imago and that of his death (Jm) was calculated. The average duration of the cycle is defined as the time elapsed between egg-laying to the adult stage. It includes incubation period (Di), larval period (Dl) and the pupal period (N). To determine the average duration of the cycle, the eggs hatched were followed until the emergence of the imago.

$$\text{Longevity of adults (days)} = \frac{\sum (Jm - Je)vi}{\sum vi} \times 100$$

vi: number of males and females

$$\text{Life cycle duration (days)} = \frac{\sum JiKi}{\sum Ki} \times 100$$

Ji = Di + Dl + Dn; Ki: number of adult

Statistical analyzes

Statistica Version 7.1 software has been used for statistical analysis. The data obtained were subjected to statistical analysis using the ANOVA test.

Results

Egg laying behaviour, fecundity, fertility and incubation period of eggs of R. phoenicis

Before the eggs are laid, the female ensures with her rostrum that the support is in good condition. After this step, she digs a hole or box of about 1.5 ± 0.5 mm of depth using its rostrum and then turns around and makes out its ovipositor in the direction of the hole to lay eggs. The eggs are laid individually in the little hut, which are closed after the eggs have been deposited with a fecal plug. Female lifetime fecundity under rearing conditions was 252.26 ± 3 , 61 eggs (range 90–288). The daily fecundity was 15.11 ± 3.64 eggs per female. Per cent fertility was $88.30 \pm 2.04\%$. The oviposition period was 29 ± 3.1 days range from 17–36 days. The incubation period of eggs of *R. phoenicis* was observed between 3-5 days with an average of 4.09 ± 0.53 days. Eggs are oval, elongated, white and measures 1.2 ± 0.2 mm in length and 0.9 ± 0.1 mm in width (Fig. 3). This color changes from white to opaque yellow 2.04 ± 0.31 days before hatching.

Table 1. Morphometric measurements of different grub stages of *R. phoenicis*.

Stages	Weight (g) (mean±SE)	Length (mm) (mean±SE).	Mean head capsule width (mm)
First instar	0,08 ± 0,09	8,96 ± 0,74	1,66 ± 0,39
Second instar	0,49 ± 0,31	18,62 ± 1	3,81 ± 0,19
Third instar	0,98 ± 0,24	30,6 ± 0,12	4,8 ± 0,24
Fourth instar	1,86 ± 0,38	37,13 ± 0,9	5,51 ± 0,17
Fifth instar	2,95 ± 0,62	40,23 ± 1,06	6,13 ± 0,03
Sixth instar	3,59 ± 0,41	51,52 ± 0,81	7,81 ± 0,62

Larval instars of R. phoenicis and development

Larval life passes through six distinct stages alternated by different molts (Fig. 4). In *R. phoenicis*, the process of molting in the larval stage begins at the level of the head capsule for all stages. The larva begins its molt through a slit in the dorsal part of the head capsule by contracting the rest of the body rhythmically until exuvia is completely removed, this process takes about 10 to 15 minutes (Fig. 5).

The cephalic capsule and the body of the newly emerged larva have a white color. It becomes light orange brown when it tends to the next molt (Fig. 6). The width of the head capsule, that of the body and the weight of the larva evolve with age (Table 1). Generally, developmental period of each larval instar was progressively longer than the preceding instar (Table 2). The total duration of larval development is $33,24 \pm 1.4$ days.

Larval survival rates, pupal development and emergence rate of R. phoenicis

Larval survival rate was 82.66% for first instar larvae and 86.15% for sixth instar larvae.

The lowest survival rates were observed in 2nd and 3rd instar larvae (Table 3). After the last molt, sixth instar larvae stopped feeding to build their cocoons. Prepupa is contracted, less mobile except for a few rotatory movements of the abdomen (Fig. 7). The prepupal period was 4.91 ± 0.56 , ranged 3-5 days.

Table 2. Duration of different stages larval of *Rhyncophorus phoenicis* at 27.86 ± 0.5 ° C, and $74.15 \pm 2.2\%$ relative humidity (RH).

Stages	Sample	Duration (days) (mean±SE)
First instar	300	4,3 ± 0,2
Second instar	248	5,13 ± 0,53
Third instar	172	5,7 ± 0,33
Fourth instar	101	5,9 ± 0, 29
Fifth instar	78	6,6 ± 0,82
Sixth instar	62	6,3 ± 0,53
Total		33, 24 ± 1.4

The pupa is yellowish-white or brownish depending on whether one is at the beginning or at the end of pupal development. It is weighed on average 2.6 ± 0.2 g. The winged sheaths are visible on the dorsal side. Head with the rostrum, eyes and antennae are visible on the ventral side (Fig. 8). At the level of the thorax, the drafts of the feet can be perceived. Pupal period was 19.3 ± 0.6 days (range 10-23 days).

The imago newly emerged from the pupal development has a color of orange that is adorned with black and finished his coloring approximately 7.4 ± 0.3 days before emerging (Fig. 9E).

The new adult emerged is black, with orange striped over the whole body (Fig. 9F). The rate of emergence of adults was 87.3%.

Table 3. Larval survival rates of *R. phoenicis* to 27.86 ± 0.5 ° C and $74.15 \pm 2.2\%$ relative humidity (RH).

Stages	Sample	Larval survival rates (%)
First instar	300	82,66
Second instar	248	69,3
Third instar	172	58,72
Fourth instar	101	77,22
Fifth instar	78	79,48
Sixth instar	62	86,15
Pre-pupa	56	85,71

Sex ratio, adults' longevity and duration of the life cycle

The sex-ratio was of 55.2% in favor of females. Female longevity was 54.71 ± 2.7 days (range 23–66). Male longevity was 68.86 ± 3.3 days (range 27–78).

The analysis of variance indicates that there is a significant difference between the life span of adults ($F=28.08$; $P < 0.05$).

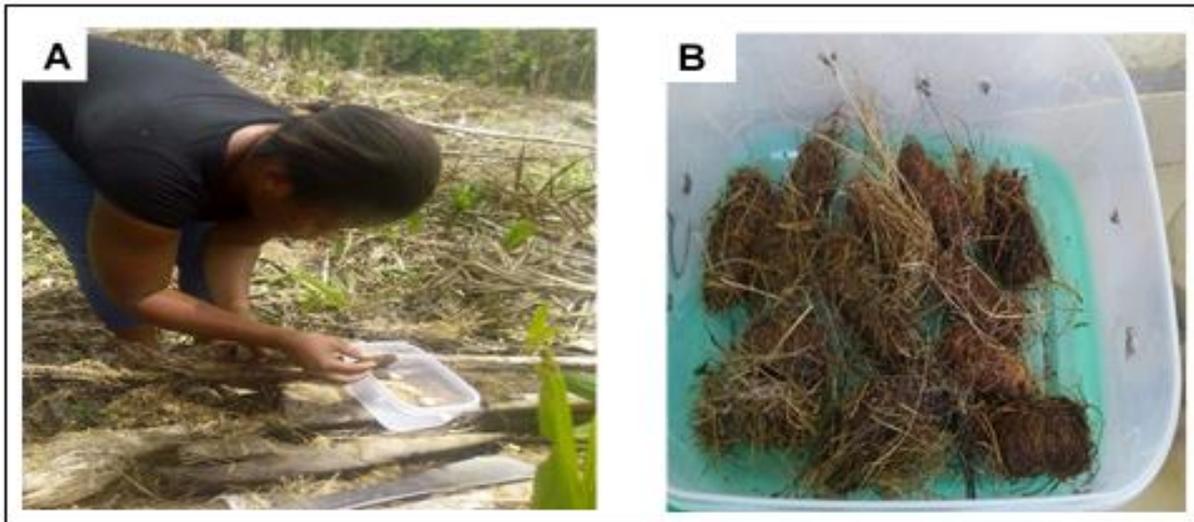


Fig.1. Collection (A) and storage (B) of cocoons of *R. phoenicis* in the field.

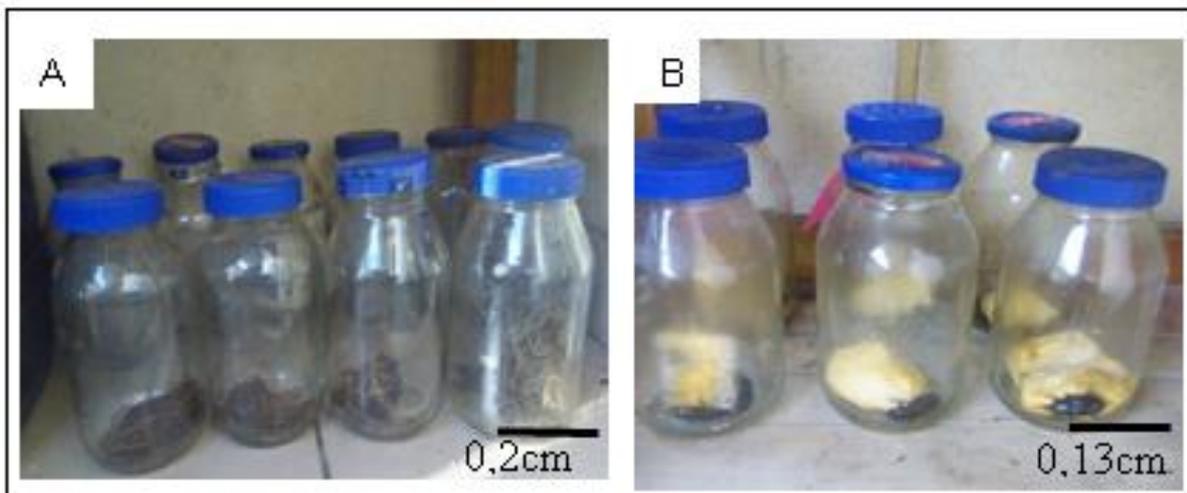


Fig. 2. Rearing of *R. phoenicis* in the laboratory: A: Conditioning cocoons of *R. phoenicis* in the laboratory; B: pairing of adults emerging from the cocoons.

The total duration of the cycle from egg to adult of *R. phoenicis* obtained in this study is to 108.51 ± 3.6 day (Fig. 10).

Discussion

In the present study, some biological parameters of the caterpillar of the palm tree *R. phoenicis* were studied to see the possibility of domestication of this insect. In the laboratory, female lifetime fecundity of *R. phoenicis* (252.26 ± 3.61 eggs) was close to that obtained by Aziza *et al.*(2013), but was higher than that obtained Mahmoud *et al.* (2015) in *R. ferrugineus* (209.3 eggs). Yong *et al.* (2015) and

Salama *et al.* (2009) found higher numbers of 342.3 ± 0.97 and 338 ± 37.24 eggs respectively in the same species on Sago in Malaysia and banana in Egypt.



Fig. 3. Freshly laid egg of *R. phoenicis* in the plam.

Per cent fertility in *R. phoenicis* ($88.30 \pm 2.04\%$) is close to those observed by Sharaby and Al-Dhafar (2013) (87 %) on sugar cane and Salama *et al.* (2009) (85 %) in *R. ferrugineux*. Yong *et al.* (2015) have recorded a fertility rate lower for this same species

(52.4 percent) on Sago palm. Incubation period recorded was 4.09 ± 0.53 days. Similar results were obtained in *R. ferrugineux* (4.5 ± 0.65) by Sharaby and Al-Dhafar (2013) and Salama *et al.* (2009).



Fig. 4. Grub stages of *R. phoenicis*: (A) first instar to sixth instar (B).

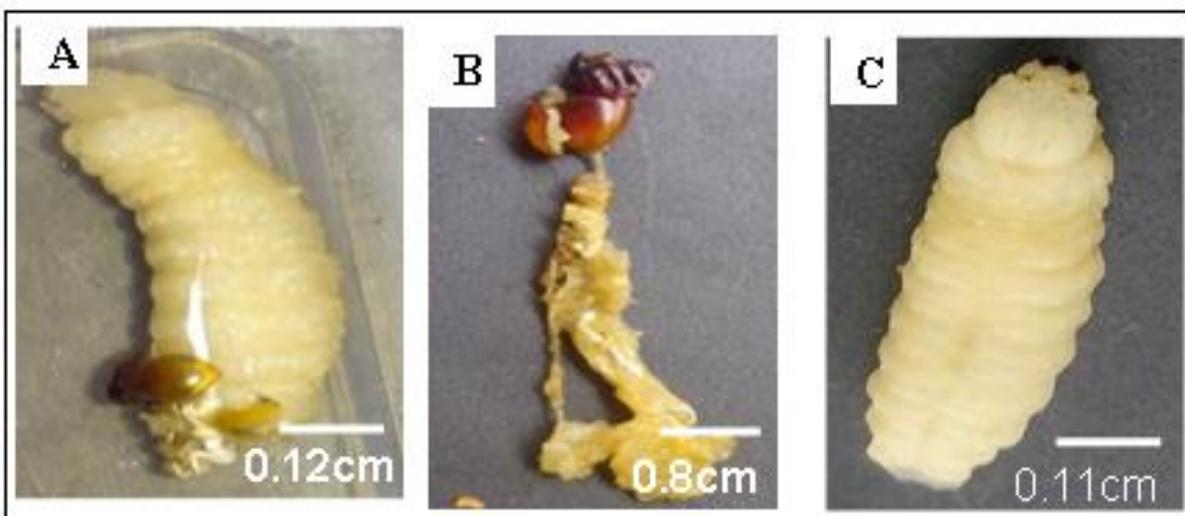


Fig. 5. Larval moulting process in *R. phoenicis*: A: larva getting rid of his old exuvia; B: larval exuvia after moulting; C: Larva having finished its moult.

The number of instars as determined in the laboratory were six as earlier reported Valdés Estrada *et al.* (2010) who have also observed six and eight instars in *Scyphophorus acupunctatus*, a species of the same family. By contrast, in the month of September of the same year, the authors determined eight larval stages, with a period of 54.2 days. The larval period in *R. phoenicis* ($33, 24 \pm 1.4$ days) is

close to that found by El-Shafieet *et al.* (2013) (36.06 ± 3.24 days) in *R. ferrugineux*. Prabhu and Patil (2009) recorded a larval period ranged from 32 and 60 days in the same species. In *R. palmarum*, this period required 60 days (Zagatti *et al.*, 2017).

The variability of larval period could be explained by the nature of the host plants. Ju *et al.* (2010) reported

that with appropriate host plants, larvae may have less stage and thus their development may be shortened. The number and nature of molts can also be affected by external factors, mainly temperature, diet and their interaction (Stamp, 1990, Wang *et*

al.(2011). The lowest larval survival rates were obtained for 2nd and 3rd instar larvae. According Tano *et al.* (2011), the survival of first instar larvae depends on the nutritional background.

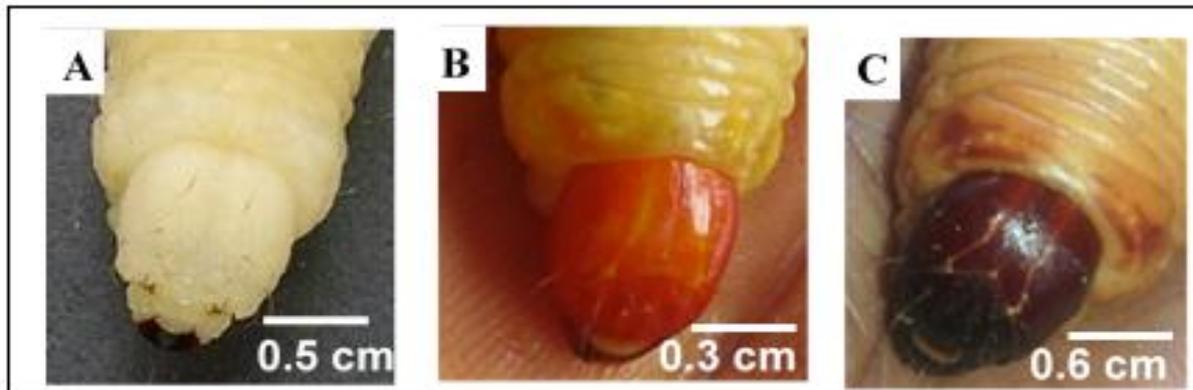


Fig. 6. Different colors of the head capsule observed in the larvae of *R. phoenicis*, A: the newly emerged, B: Five hours after moulting, C: 24 hours after moulting.



Fig. 7. Pre-: -pupa of *R. phoenicis* (A) profile and dorsal view (b).

The duration of nymphal development (19.3 ± 0.6 days) obtained in this study is in agreement with the results estimated by Aldhafer *et al.* (1998) where nymphal period was between 19 and 26 days at *R. ferrugineus*. The emergence rate of adults was 87.3% in *R. phoenicis* this value is higher compared to that obtained by Aziza *et al.* (2013) (75.5%). In this study, the sex ratio was in favor of females, these results agree with those of Mahmoud *et al.* (2015) during the rearing of *R. ferrugineus* on three different substrates. Mean longevity recorded in this study show that males (68.86 ± 3.3 days) lived longer than females (54.71 ± 2.7 days).

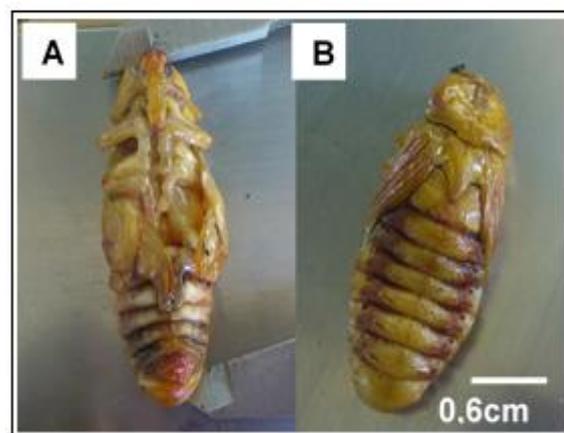


Fig. 8. Pupa of *R. phoenicis* (A) Ventral and (b) Dorsal view.

This observation is confirmed by the results of Salama *et al.* (2009) who recorded a longevity of 39 days in males and 38 days in females of *R. ferrugineus*. The

total life cycle time from egg to adult *R. phoenicis* obtained in this study was 108.51 ± 3.6 days.

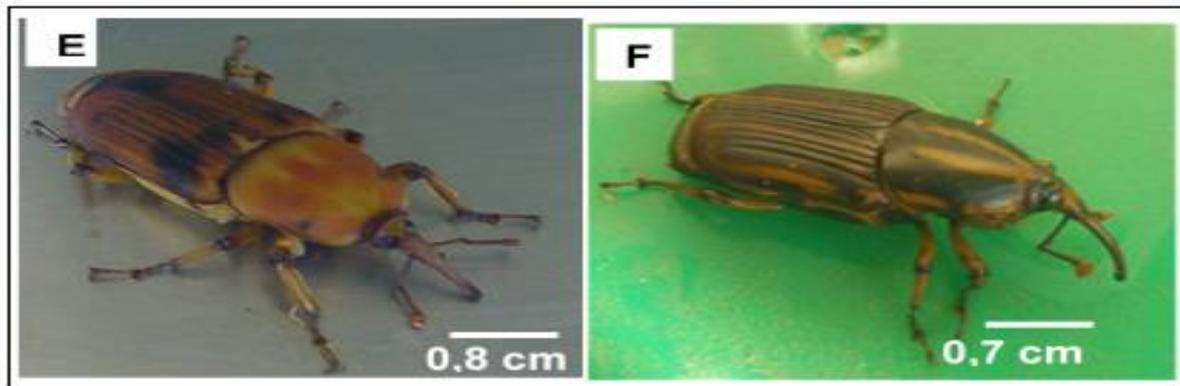


Fig. 9. Pre-imaginal (E) and imago of *R. phoenicis* (F).

This duration is very close to that of Sharaby and Al-Dhafar (2013) which was 108.8 ± 0.45 days in *R. ferrugineus* on the basis of the palm tree. On the

other hand, Abbas *et al.* (2013) and Mahmoud *et al.* (2015) noted that the length of the *R. ferrugineus* life cycle is approximately 3-4 months in the laboratory.

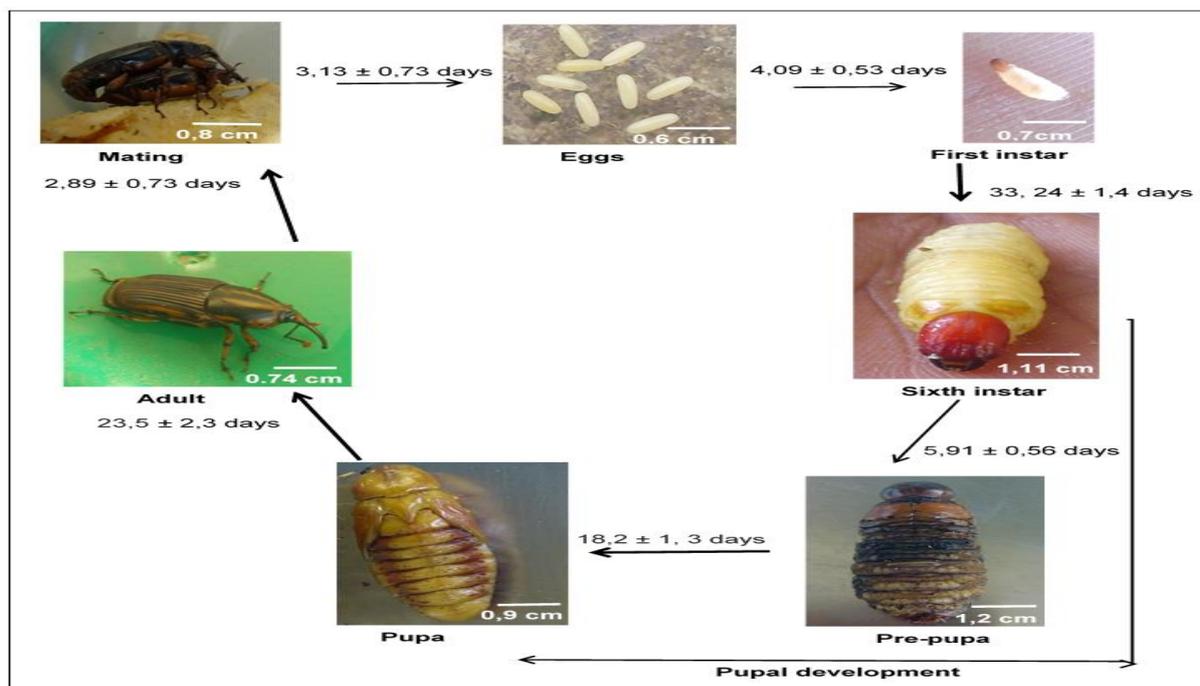


Fig. 10. Life cycle of laboratory reared *Rhyncophorus phoenicis* Under 27.86 ± 0.5 °C and $74.15 \pm 2.2\%$ relative humidity (RH).

Conclusion

This study shows the possibility of raising the *R. phoenicis* palm weevil in a controlled environment. Female lifetime fecundity was 252.26 ± 3.61 eggs per female and percent fertility was 88.3%. Six larval stages were identified with a total duration of $33, 24 \pm 1.4$ days.

Sex ratio was 55.2% for females. Emergence rate of adults was 87.3%. Males live longer 68.86 ± 3.3 days than females 54.71 ± 2.7 days. The duration of the development cycle is 108.51 ± 3.6 days. This information indicates that it is possible through breeding to supply the markets continuously throughout the year.

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