

Biology of Red Palm Weevil on Different Date Palm Varieties under Laboratory Conditions

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Abstract

Background objectives: The date palm, *Phoenix dactylefera* is one of the main crops of upper Sindh attacked by Red palm weevil known as an economically important tissue-boring pest, causing serious damage to tree trunk. It was necessary to conduct research over the biological parameters of the pest such as; egg, larva, pupae and adults with concern of colour, incubation period, hatching %, life span on different varieties, cocoon formation, male and female, (♂:♀) longevity, mating behavior, morphological characters of all stages under laboratory conditions.

Methodology: Biology parameters have been done under the laboratory conditions on the three varieties of date palm namely Aseel, Fasly and Karbalian, and then results were compared through ANOVA to check the significant results.

Results: The results of biology indicated that Aseel date palm was the most suitable variety for development of all stages from oviposition to the development of all larval stages shortest life cycle recorded on it. Aseel variety was best medium for egg laying; highest number of eggs was laid on Aseel (230) and lowest on Karbalian, (175). Highest hatching percentage was observed on Aseel 85% and lowest on Fasly 72%. Larval growth was observed best on Aseel variety (82%-92%) lowest on Fasly variety (72% to 82%). The biology of red palm weevil from egg hatching to the development of larval stages was high on Aseel variety than all remaining varieties which means it causes serious damage to Aseel.

Conclusion: It was observed the 7 larval stages on 3 date palm varieties for 3 successive generations, comparatively the Fasly variety found less suitable for the egg, larval development and shorter life span of both sexes, respectively.

Keywords: Life cycle; *R. ferrugineus*; Different varieties; Aseel; Fasly; Karbalian; *P. dactylefera* and Laboratory conditions

Significant statement

1. The many orchards are being destroyed by this vigorous pest and growers got huge loss since appearance of the pest. Such biological research parameters were also kept under observations for its proper management purpose.
2. The egg lying, time duration, hatching percent, all frequent stages of pest up to adults were kept under observations for checking the life time duration.
3. The larval stages are the most damaging to the date palm so the management techniques were kept in mind as well.
4. The male and female (♂:♀) differences with mating behavior, duration and justification of the red palm weevil pest with its morphological characters were also focused during the research as well.
5. There were so many different date palm varieties above the (250) the main and commonly growing varieties (Fasly, Aseel and Karbalian) were kept under observations.

Introduction

Date palm, *Phoenix dactylefera* (L.) belongs to the family Arecaceae which is considered as an important cash crop of district Khairpur, which is also the major dates producing district of Sindh. The Pakistan is ranking in 3rd country after Iran and UAE, in date-exporting in the world. According to export promotion bureau, the export of dates during, 2000-01 surpassed 75,000 tons. The world production of date 535000 tons annually with growth of 166% right throughout in last three decades but the production of Pakistan was fall down due to certain reasons. Date palm is known as an important cash crop of Sindh, Pakistan. In dates the Aseel variety is cultivated mostly in Khairpur, Sindh and Punjgoor, Balochistan with it's an internationally importance. As per conservative estimate, the total land under date-palm in Khairpur is about 100,000 acres. Total manufacture of dates in Pakistan reportedly stands at 293,000 tons of which Sindh shared 37% Balochistan 32% and reaming belongs to Khyber Pakhtoon Khawa, Punjab [1]. Very minute efforts have been made in Pakistan to control the possible of this yield. The main dates growing areas are Khairpur, Nawabshah, Kotri, Rohri, Bannu, Punjgoor, Dera Ghazi Khan, Bahawalpur, Multan, Turbat and Dera Ismail Khan. More than 600 varieties of dates are being cultivated throughout the world. It is affected by many insect pests out of these Red palm weevil is the major pest that affects the crop of date palm in Sindh [1].

The red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) is an economically important, tissue-boring pest of date palm in many parts of the world. The insect was first described in India as a serious pest of coconut palm [2] later on; it was also observed on date palm [3,4]. It is a polyphagous pest and distributed worldwide mostly in tropical and sub-tropical parts of the world. Various studies have been conducted on red palm weevil mostly these were conducted in India and South East Asia from 1900 to 1950 [5]. The larvae of red palm weevil damage the date palm tree by a bore in its soft tissues of the trunk region in the growing period of the tree [6]. For oviposition, female use their rostrum to bore into the tissue and forming holes, in which they lay their eggs, [7] reported the red palm weevil takes shelter and lay eggs under the splitting bark and newly emerging roots. In several attack the larvae of date palm weevil makes cavities inside tree.

It is further described by [8] that the variety Aseel of date palm has high sugar content material because of which attracted by this vigorous pest, who conducted the studies over ecological and biological studies on the red palm weevil, *Rhynchophorus ferrugineus* (Olivier). Many research workers have been working and summarized the developmental stages of RPW [5]. The development of red palm weevil, *R. ferrugineus* was successfully reared on the sugarcane stem [9], while standardized a mass rearing method for this pest from which 50 generations were obtained subsequently [10]. The developed an artificial diet for rearing the weevil using sugarcane bags [9], fresh coconut cake, brewers, yeast, sugarcane potassium hydroxide methyl benzoate and sorbic acid solution. Further, [8] reared the insect on a semi artificial diet in Egypt, while [11] developed an artificial diet for RPW consisting of potato and vitamins B and D, [12] reared red palm weevil on four major cultivars of dates, such as; Sillaj, Khasab Khalas and Sukkary (local names of varieties) [13] did a very extensive work on the development of RPW, he did a laboratory mass rearing on the stem of sugarcane, prior to mass rearing, he used several artificial diets such as oats, coconut cake, coconut fruit pieces, canned and fresh pine apple, sucrose, molasses, egg yolk salt, yeast, vegetable oil, potatoes, soybean flours, date palm leaves and palm fiber sheath sugarcane fiber for the life cycle of *Rhynchophorus ferrugineus*.

The red palm weevil as a foreign enveloping pest of date palm, [14,15] worked on the control of red palm weevil [16] reared red palm weevil in laboratory and also in the field conditions on sugarcane and pieces of apple and artificial diets 20% honey solution. Being a main source of a big population of upper Sindh who directly/indirectly depend on the production of dates for their lively hood, and because of its nutritional value and also one of the main source of foreign exchange there was a great need to do some work on the pest problem of Red palm weevil, which is causing severe damage to our one of most economically important crop. Presently here we tried to do work on major aspects of Red palm weevil, biology in the laboratory under the controlled condition, as to get some basic information about time period of the life cycle (study the different larval stages, which are actually the most destructive stages) on three main varieties, Aseel, Fasly and Karbalian (commonly grown in Khairpur and other parts of upper Sindh), which are directly co-related to the damage. This type of study will ultimately lead us to the control of this destructive pest. This would be helpful in controlling the Red palm weevil problem and can increase the dates yield/hectare.

Materials and Methods

Collection of insect: Different life stages: adult red palm weevil (male and female), larvae and pupae were collected from infested date palm trees from different localities of district Khairpur Mir's, Sindh, Pakistan such as; (Hussainabad, Kot degi, Thari Mirwah, Piryalo). We have collected all the life stages preferably from infested trees of date palm of some commonly grown varieties of district Khairpur, such as; Aseel, Fasly, Karbalian, Red and Khopro, but for on study in the laboratory we have selected most popularly consumed three varieties Aseel, Fasly and Karbalian. The collection of adult and larvae of Red palm weevil was took place by hand picking from tree trunk. We kept the specimens of red palm weevil separately variety wise in jars, for culture with their natural food and labeled them. Rearing of red palm weevil was conducted in Entomology laboratory of Zoology department under controlled condition on their natural diet date palm on 25°C and humidity 60-70% except cocoon which reared 29°C. We used incubator for temperature control, for incubation.

Identification of male and female: After collection from field the adult male and female brought into laboratory for identification and rearing. The male had a patch of brown hairs on half of rostrum, while female was without patch of brown hairs. The male were relatively shorter in size than female. After identification each pair of (male and female) kept in separate jars, measured 3 × 4 inches and provided them stem piece of date palm tree of all three varieties, Aseel, Fasly and Karbalian which measured (size 6 cm long) and weight is 50 gram. These jars were placed in a plastic box of size 30 × 20 inches contained salty water, temperature was maintained at 25 ± 2°C and 60-70% humidity. The mating and egg laying took place in these jars. The cap of jars was covered with muslin cloth for ventilation.

Medium for egg hatching: Eggs were shifted equally with the help of fine brush from jar to Petri dishes with piece of stem of different varieties of date palm for hatching purpose. One Petri dish was lined with the moist filter paper and a piece of stem Aseel size of 3 × 2 cm as well as to dry filter paper with piece of stem of Aseel size 3 × 2 cm. One Petri dish was lined with the dry filter paper with piece of stem of Fasly size 3 × 2 cm. One Petri dish was lined with the dry filter paper with piece of stem of Karbalian size 3×2 cm as to observe the percentage of hatching on different mediums.

Development of larvae: After hatching from eggs theses larvae were transferred in jars with the help of fine hair brush and these jars contained the diet with a piece of stem of Aseel variety (size 5 × 3 cm), piece of stem of Fasly variety (size 5 × 3 cm) and piece of stem of Karbalian variety (size 5 × 3 cm). These jars were kept in plastic box (30 × 20 inch); development from first larval instars to adult took place in these jars. As the larvae changed the instars they are separated in to jar for observing complete molting as observe the changing in the instars. We observed daily and changed stem piece and providing fresh pieces as well.

Pupal stage: For pupation of Red palm weevil we provided a piece of date palm stem, to (last larval instars) because they required fiber of trunk for cocoon making. Without making fiber it was also observed that the larvae failed to make cocoon, after pre-pupal stage they form cocoon inside the stem piece after 5 days the stem pieces were cut down longitudinally with careful handing for the cocoons collection. These cocoons were shifted in separate jars. These jars were kept in plastic boxes at 29°C temperature and the emergence of adult took place in these jars.

Feeding behavior: For observing feeding behavior, a piece of 6 × 5 cm size and 50 germ weight of each variety (Aseel, Fasly and Karbalian) were provided to each larval instar (1st-7th) separately. The given pieces were changed weekly and the size and weight of infested stem piece was also measured by Electronic balance. This whole process was continued for three successive generations and data was collected and analyzed to observe the percentage of damage and monitor the varietal susceptibility of date palm against Red palm weevil. Finally, the data was analyzed for its checking the significant results through ANOVA, for this purpose SXW software, 8.1 version (USA) was used.

Morpho-taxonomic appearance of different life stages, egg, larvae, larval stages, cocoon formation, adult (♂:♀) and mating behavior under laboratory conditions



Figure 1: Eggs of RPW.



Figure 2: First instars larvae after hatching.

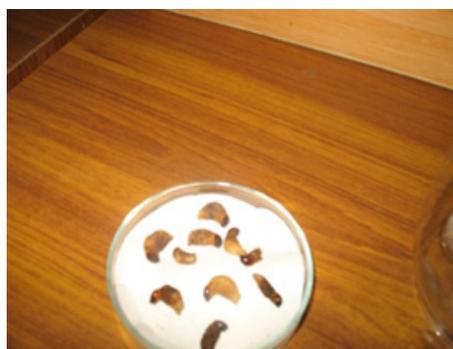


Figure 3: Different larvae stages in Petri dish.



Figure 4: Cocoon which posse's pupae.



Figure 5: Adult of RPW emergence from cocoon.

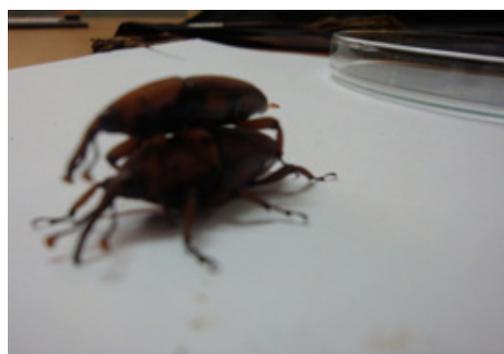


Figure 6: Mating behavior of Red palm weevil (♂:♀).

Results

Biology under laboratory conditions

Incubation of eggs: Newly laid eggs were yellowish white in colour, measured about 2.5 mm long. Eggs hatched after 3 to 6 days, we kept 110 eggs of Red Palm Weevil on each variety of date palm in Petri dish, lined with moist filter paper, 90 eggs were hatched on Aseel and 94 eggs of same variety hatched in Petri dish lined with dry filter paper. The number of eggs hatched on Fasly was 80 on (dry filter paper) while 84 eggs were hatched on Karbalian variety on (dry filter paper) [Table 1]. The results indicated there was no significant difference on egg hatching on all three varieties but Aseel looked favorable medium (94%) for egg hatching at ($P < 0.05$).

Egg Hatching substrate	No of eggs observed	No. of eggs hatched	Hatchability (%)
Moist filter paper with Aseel	110	90	81.8
Dry filter paper with Aseel	110	94	85.4
Dry filter paper with Fasly	110	80	72.7
Dry filter paper with Karbalian	110	84	76.3

Each value of a means in column are significantly different at ($P < 0.05$).

Table 1: The hatchability percentage of *R. ferrugineus* on different date varieties on filter paper.

Development of Larvae

First instars: The red palm weevils are borer of date palm trees so they remained inside the given pieces of stem, and kept feeding on soft pulp. The maximum survival rate of first instar was high on Aseel, about 85.4% and was 76.3% on Karbalian and 72.7% was recorded on Fasly [Table 2]. The whole body of first larval instars was yellowish white in colour, except head which was light brown, segments of body was very visible, the body possessed no appendages. The whole body was soft and delicate; they fed only on soft tissues of date palm trunk. The first instar larvae measured 2.5 mm [Table 3]. There was no major difference in the life span of first larval instars on all three varieties; it was 12 days on Aseel, and Karbalian and 13 on Fasly.

Second instars: The results indicated that the maximum survival rate of second instars was almost same on two varieties with minor variation on Aseel and Karbalian 86.1% and 85.7%, while the lowest was on Fasly 80% (Table -2). The second larval instars also looked same except the visible difference in the length of body; body colour was yellowish white and head was light brown, segments of body was very visible, those measured about 10.5 mm [Table 3]. Total life period was recorded 13 days on Aseel, 15 on Fasly and 14 days on Karbalian.

Third instars: Like, 1st and 2nd instar the body of third larval instar was also soft so they fed on soft tissues of date palm. The larvae, from second larval instar to third larval instar were same in colour and shape, except the size which was variable in each instar. The survival rate of third instar was almost same on Aseel and Karbalian 83.9% and 83.3%, while on Fasly it was distinctly less about 75.1% [Table 2]. The third larval instars were also pale in colour and head was light brown, segments of body were very visible. Length of body larger than 2nd instar measured 14.6 mm distinct variation was recorded in life span of 3rd instar reared on three varieties of date palm. Total life period of third instars recorded 11 days on Aseel and 13 days on Karbalian while 14 on Fasly [Table 3].

Fourth instars: They fed on soft tissues of date palm, the maximum survival rate of fourth instar was observed 82.3% on Aseel and 81.6% on Karbalian and 74% on Fasly [Table 2]. The fourth larval instar was also pale in colour but head turned in little dark brown, segments of body were very visible, like other instars the size of fourth instar's increased that measured 20.9 mm [Table 3]. Total life period of fourth instars was 10 days on Aseel, 12 days on Fasly and Karbalian.

Fifth instars: The body of fifth instars is relatively harder than first fourth instars, the mouth parts also started developing, became stronger than first four instars so they started feeding on harder parts of stem too. The maximum survival rate of fifth instars was 87.5% on Aseel, 85.7% on Karbalian and minimum was recorded on Fasly about 78.3% [Table 2]. In the fifth larval instar the body colour started changing and it was yellowish pale, but the head was almost same brown, segments of body was very visible as of fourth instars. The size increased, it measured 30.15 mm [Table 3]. Over all no significant difference was recorded in the life span of fifth larval instar on all three varieties ($P < 0.05$); it was 09 days on Aseel, 11 on Fasly and 10 days on Karbalian.

Sixth instars: Body and mouth parts got stronger than fifth instar so they started feeding on interior tissues of the stem and made a visible hole in the center of stem piece. The maximum survival rate of sixth instars, like previous, was almost same on Aseel and Karbalian, 91.8% with 90.4% and minimum was on Fasly 79.3% [Table 2]. The sixth larval instar was pale yellow like fifth instar but unlike

first five instars here in sixth instar head became darker and turns dark brown, segments of body was very visible. The sixth instars larvae were much larger than the previous instars and measured, 40.22 mm [Table 3]. Total life period of sixth instars recorded 9 days on Aseel, 10 on Fasly and 11 days on Karbalian.

Seventh instars: The body of this full grown up larvae was much harder than all other instars and mouth parts were well developed therefore they were feeding very fast and produced the frass (chewed up plant fiber) in a big amount that caused severe damage to the plant trunk; seventh instar was the last stage of larvae, later on it turned into pre-pupal stage that was inactive period. The maximum survival rate of seventh instars was observed 88.6% on Aseel, 86.8% on Karbalian, and 82.6% on Fasly [Table 2]. There was a visible difference in the body colour, structure and size of seventh instars. The body of seventh larval instars was relatively darker, looked brownish yellow with head dark brown, segments of body was very visible. It was very long and measured 49.28 mm [Table 3]. The life period of 7th larval instars were same, 8 days on Aseel and Karbalian but distinctly longer on Fasly, about 11 days.

Larval instars of RPW	Aseel (%)	Karbalian (%)	Fasly (%)
1 st	85.4	76.3	72.7
2 nd	86.1	85.7	80.0
3 rd	83.9	83.3	75.1
4 th	82.3	81.6	74.0
5 th	87.5	85.7	78.3
6 th	91.8	90.4	79.3
7 th	88.6	86.8	82.6

Each value of a means in column are significantly different at ($P < 0.05$).

Table 2: The mean number of larvae survived on different date varieties (Aseel, Fasly, and Karbalian).

Larval instar	Aseel (days)	Fasly (days)	Karbalian (days)	Measurement (mm)
1 st	12	13	12	02.5
2 nd	13	14	14	10.5
3 rd	11	14	13	14.6
4 th	10	12	12	20.9
5 th	09	11	10	30.15
6 th	09	10	11	40.22
7 th	08	11	08	49.28

Each value of a means in column are significantly different at ($P < 0.05$).

Table 3: Average larval duration and measurement of *R. ferruginus* fed on different varieties of date palm.

Pupa: The seventh larval instar stopped feeding for 2-3 days in pre-pupal stage and then started spinning cocoon around its body from the fibers of damage trunk; the spinning of cocoon completed in 5 days; the cocoon was approximately 35-80 mm long. The pre-pupal period was recorded 2 to 3 days on Aseel and Karbalian and 2 to 4 days on Fasly. The minimum pupal period was recorded on Aseel was 14 to 21 days and maximum on Fasly and Karbalian 18 to 30 days [Table 4].

Adult: After emergence from pupa, the adult started feeding to hard tissues of stem pieces, adult was large in size with highly sclerotized body and long slender like rostrum (snout) that helped in penetrating into stem pieces. Male and female apparently look alike except that male has patch of brown hairs on half of rostrum, while female was without patch of brown hairs with the life span of female was relatively less than male. Aseel looked most favorable host for adult; the results showed that the longevity was distinctly more on this variety than other two varieties. Maximum longevity time of male was recorded on Aseel, about 70-110 days and of female, was 65-100 days; on Fasly about 50-90 days of male and female life span was 50-82 days; the shortest life span of male was recorded on Karbalian, about 50-82 days and of female 55-90 days [Table 4].

Complete life cycle: There was a significant variation in maximum time of total life cycle duration but minimum time was almost same about 30 days on all three varieties. The life cycle was in 30 up to 110 days on Aseel variety of date palm that was completed in 30 up to 100 days on the Fasly. The life cycle was completed in 30 up to 120 days on the Karbalian variety of date palm.

Life stages	Aseel in days	Fasly in days	Karbalian in days
Incubation period	4-5	5-6	4-5
1 st	12	13	12
2 nd	13	14	14
3 rd	11	14	13
4 th	10	12	12
5 th	09	11	10
6 th	09	10	11
7 th	08	11	08
Pre-pupal period	2-3	2-4	2-3
Pupal period	14-21	18-30	18-30
Male longevity	70-110	50-90	55-95
Female longevity	65-100	50-82	55-90

Table 4: Mean incubation period, pre-imaginal growth and adult longevity of *R. ferrugineus* supplied with different date varieties.

Mating, egg laying and biology behavior: After emergence from pupae, we have collected three pairs (male and female) of Red palm weevil and kept them in three separate jars with stem pieces of one of these three varieties of date palm (Aseel, Fasly and Karbalian). Mating process took place at the beginning of night, usually with the sun-set. The whole process completed in one to two minutes. After mating female made holes inside the piece of stem and started egg lying singly in each hole. Usually, this egg laying process for one batch took place in 2-3 days [Table 5]. The biology revealed that among three varieties, Aseel date palm (150-230) was the most affected variety because the female found it most suitable for oviposition and the development of larval instars was also very fast followed by Fasly (125-190) and Karbalian (115-175), respectively.

Name of the varieties	No of eggs deposited
Aseel	150-230
Fasly	125-190
Karbalian	115-175

Table 5: The mean number of eggs laid per female on different varieties of dates.

Discussion

Red palm weevil, *Rhynchophorus ferrugineus* being a serious tissue borer pest of date palm trunk in upper Sindh, causing severe infestation to the tree, made it weak, therefore ultimately it affected the date production. Therefore we have tried to do some research on this serious pest and got some figures/data about its presence and mode of biology to observe the variation in the developmental time on three main varieties of dates (grown in upper Sindh). The research conducted by [16] who described the frequent distribution and damage of RPW, *Rhynchophorus ferrugineus* (Oliv.) (Coleoptera: Curculionidae) by using ecological niche modeling technique, it is in agreement with our research study that found this pest is present where, there is date palm orchard, known as most devastating pest of the globe, and there control measure techniques were also described accordingly by [17], how it reacts with the immune system [18] and the biological parameters [19] of this vigorous pest, respectively.

During present study we have recorded seven larval instars on its natural diet (all pieces of date palm trunk of three varieties), most of researches like [20] also recorded same number of larvae but [17] had different findings and recorded nine larval instars on artificial diet and sugarcane. The time period of larval development was significantly different on three varieties; our results of three successive generations showed that varied from 1 to 3 months, on all three varieties Aseel, Fasly and Karbalian. The development rate of larvae was observed high on Aseel than Karbalian and Fasly, we assumed that it was because of the more sugar content in Aseel; it was sweeter than other varieties. It had same kind of observations [8] have reported that red palm weevil was successfully reared on diets having high sugar contents. The minimum period of development, 1st to 7th larval instar was recorded on Aseel, which was about 45-80 days, succeeded by Karbalian 50-80 days and maximum developmental time of larva was on Fasly, about 55-90 days.

The results of present study indicated that Aseel was the most susceptible to both adult and larvae of date palm weevil; we have recorded the shortest larval developmental time on Aseel about 45-80 days, the larvae have eaten the inside pulp/fibers of given piece of Aseel stem voraciously, made it almost hollow. We assumed that as the fibers of tree trunk of Aseel were moist and soft so the first larval instars could feed easily, which left positive effect on the growth rate of larvae and development got quite faster than other two varieties. After Aseel the second most infested variety was Karbalian, and the least was Fasly. Significant differences in the duration of all life parameters of *R. ferrugineus* on different diets were found. These included oviposition period, larval and pupal periods, adult male and female development times, and generation span. Significant differences were observed in the egg laying, reared on different diets, ranges from 3-4 days on both Aseel and Karbalian variety of date palm and 3-5 days on Fasly variety of date palm, these results agreed with other researchers like [13] and [21,2,16] all reported 3-5 days. While our results were not compatible with the some researchers reported 1-6 days, and 2-3 days as well.

Significant differences were recorded in the number of egg laid on all three date palm varieties ($P < 0.05$). Aseel looked most suitable for oviposition therefore; highest number of eggs (230) laid on it, succeeded by 190 on Fasly and 175 on Karbalian variety. It had more or less same findings on their local varieties (150-250 eggs) other researcher finds variable number of eggs 531, [2]. But the percentage of hatchability (viability of eggs) was almost same with past and present researchers it ranged from 85.4% on Aseel, 76.3% on Karbalian and 72.7% on Fasly. These results agreed with [2], who recorded 87%. Our results showed the significant differences in the developmental time of larvae when reared on three varieties, ranged from 45-80 days on Aseel, 50-90 on Fasly and 50-80 Karbalian. These results agreed with the some other world researchers who reared red palm weevil on different diets (artificial and natural diets), like [22] reported 81-89 days, [23] reported 41-78 days, [2] reported 55 days and [24] reported 60 days, while some researchers reported different findings from ours, like [25] reported 60-120 days, and [13] reported 70-102 days. Life span of adult male and female was variable on all three diets; longest life span was observed on Aseel about 65-110 days while it was almost same on Fasly and Karbalian, 55-90 days and 55-95 days respectively, these results agreed with the [26] reported 50-90 days, [2] reported 60-90 days. Whereas; the same kind of work was also described but that was on saw toothed grain beetle of date palm fruits [27,28]. During present study of biology was observed among all three varieties which were positively different to each other on these date palm varieties under laboratory conditions.

Conclusion

It was concluded from this research findings that 3 date palm varieties such as; Aseel, Fasly and Karbalian (commonly grown) were selected for the pest infestation. The shortest developmental time was recorded on Aseel and longest was on variety, Fassly whereas; variety Aseel also found most suitable for oviposition. Thus, the 7th larval instars were observed longest and most infectious among all larval instars. Therefore, it is recommended that the Red palm weevil is easy to control at egg and first larval stage because these stages are sensitive, vulnerable and easy to control.

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