

Study some biological aspects of peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) In laboratory and field

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Abstract

The objective of the study is to establish the basic rules for studying the life of the insect, for subsequent studies because the insect has recently entered Iraq, study investigated the effect of temperature 20, 25, 30 ± 2 °C and field conditions on the biological aspects of peach fruit fly *Bactrocera zonata*. The results of the study showed that the shortest incubation period for eggs, larvae, pupae, pre-oviposition period, and post-oviposition period and life cycle duration was 8.7, 8.2, 10.8, 26.4, 4.2, 16.9 days respectively at 30 ± 2 °C. Moreover, the highest incubation period was 17.8, 19.3, 23.9, 43.6, 9.4 and 37.1 days respectively at 20 ± 2 °C when larvae fed on artificial larval diet. While it was 19, 13.6, 22.3, 29, 5.3, and 32.6 days respectively in field conditions when feeding the larvae on the mandarin fruit, with a statistical significant difference between temperature. The ratio of emergence was 86, 86, 68% at 30, 25, 20 ± 2 m and 63% at field conditions and with significant difference. The highest number of eggs was 218 egg/female at a temperature of 30 ± 2 °C, the lowest number was 70.8 egg/female at field conditions, and there is a statistical significant difference. The highest hatching rate for eggs was 80% at 25 ± 2 °C and the lowest hatch rate was 64% at 20 ± 2 °C. The ratio of sex (females: males) was 0.54: 1, 0.79: 1, 0.79: 1 at 20, 25, 30 ± 2 °C respectively and it was 0.58:1 in field conditions. The highest number of eggs in virgin reproduction was 83.2 egg/female at 30 ± 2 °C, and the lowest was 65.4 egg/female at 20 ± 2 °C, and hatching rate was zero. The longest age of the female in virgin reproduction was 80.4 days and the shortest 44.4 at 30 ± 2 °C, while in the field conditions 62.3 days. It is clear that the difference in temperature and environmental conditions have a significant impact on all biological aspects of the insect.

Keywords: Peach fruit fly, Biological aspects, different environmental conditions, virgin breeding

1. Introduction

Peach fruit fly (PFF) *Bactrocera zonata* (Saunders) family Tephritidae, This genus includes about 440 species, which causing significant losses to many fruits of tropical and subtropical trees. Most species are found in the tropics, South Pacific and Australia (White and Elson-Harris, 1994).

The damages that caused by PFF was reached 100% of fruit without control (Hardy, 1997). In addition to in addition of quarantine legal that prevented these fruits to imported many countries. Preventing these fruits from entering to many countries which are importing these fruits (Joomaye et al., 2000), Losses caused by the insect are estimated about 320 million \$ in the middle East and about 190 million \$ in Egypt annually (FAO/IAEA, 2000).

The Peach fruit fly *B. zonata* was apolyphagy pest which infect more than 40 vegetable and fruit crops such as guava, mango, peach, papaya and citrus fruits (Alzubaidy, 2000; Stonehouse et al., 2002).

The temperature has a significant effect on the development of eggs and eggs hatching ratio when fed on artificial larval diet made from wheat bran (Mohamd, 2009).

The length of development period instars of the peach fruit fly are decreased when high temperatures from 25 to 30 °C during laboratory rearing and relative humidity 54-65%, 14-10 (light and darkness) (Marwa et al., 2012; Khattak et al., 2012), The development period of all peach fruit fly instars decreased when high temperatures from 15 to 25 °C (Ali, 2016).

The first record of this insect in Iraq in 1972 from fruit samples imported from Bahrain (El-Haidari et al., 1972), then confirmed as peach fruit fly *Dacus zonatus* it has been infect fruits of peaches and mango, watermelon and Alfalfa, then insect disappeared from the Iraqi environment (Al-Ali, 1977). After that, *B. zonata* was recorded in orchard of Alhafrya city in Was province and caused severe damage to citrus

fruites(Abdulrazak et al., 2016) , Because of the spread of the insect in many orchards, which caused damage to many of the fruits, and in the absence of adequate studies on the insect in Iraq, this study was conducted.

2. Materials and Methods

2.1.Preparing a continuous laboratory farm of insects

To obtain different stages of insect life for use in laboratory studies. The fruits of the mandarin, identified by the infection, by egg-laying scars, partial damage and wet appearance from Baghdad's Al- Jadriya orchard, which contained a variety of fruit trees, which observed the presence of insects in advance. The fruits were taken during the first week of October 2017.

Samples were transferred to the laboratory and distributed to three plastic containers (30 x 20 x 20 cm). Sterile soil was placed in the base of plastic containers at a height of 2 cm, for the purpose of turning the larvae into pupal. Ten fruits are placed in each plastic container. Plastic containers are covered from the top with a gauze cloth and fitted with a rubber band. Daily observation was carried out to monitor larvae exit to the soil, After pupal all the larvae, the fruit was removed from the plastic containers and the soil was taken and sieved for the purpose of isolating the pupal from the soil l. The pupal placed in glass dishes that have a light layer of sterile soil. For obtaining the adult insects. The glass dishes which opened its covers were transferred to wooden cages (30 x 20 x 20) cm. One of the sides of the cage is covered with a muslin cloth; this side has a 15cm in diameter hole covered with a muslin cloth that is fixed in a long cylindrical shape, its end was tied with a rubber band. The base of the cage consists of a 1.5 mm clamp wire. Other sides of the cage are made of transparent organic plastic .The cages are placed over plastic containers filled with water to provide relative humidity to the insect. The cages kept under daily monitoring to the emergence of adult insects. It prepared the necessary nutrient medium to provide the female with the necessary protein for egg laying and to stimulate male sexual activity, consisting of one part(Yeast Hydrolysate Enzymatic) with three parts sugar in a 9 cm glass dish (Slansky and Scriber, 1985). A glass dish containing a layer of water-saturated cotton was placed in each cage. After insects emergence, some adults had been sent to the Natural History Museum / University of Baghdad which has been confirmed by Dr. Hana Hani Abdul Hussein Al-Saffar as the peach fruit fly (*Bactrocera zonata*).

2.2. Design the pot to get peach fruit fly eggs to study aspects of life

The egg-laying pot for the peach fly consists of a 300 mL yellow cylindrical plastic container. Hole the upper half of the enclosure by a 1 mm diameter pin(1-A). Use a small cylindrical container half the size of the first container and a hole in all its sides with a 1 mm pin (1-B). The lid of the small container is installed inside the lid of the large container with a small screw (1-C). A piece of tangerine is placed in the small container to be the source of the odor that attracts the insect and closes with its fixed surface with the lid of the large container (1-D). Water was added to the lower quarter of the large container and covered with a lid on which the small container was installed (1-T). Place the Egg laying pot inside the culture cage containing insect adult (female and male). The insect inserts ovipositor into the hole in the large container and starts laying eggs which fall off into the water at the bottom of the container. The eggs were collected every 12 hours by pouring out the water through a piece of gauze to isolating the eggs and used in later experiments. The Mandarin fruit was replaced every 24 hours.

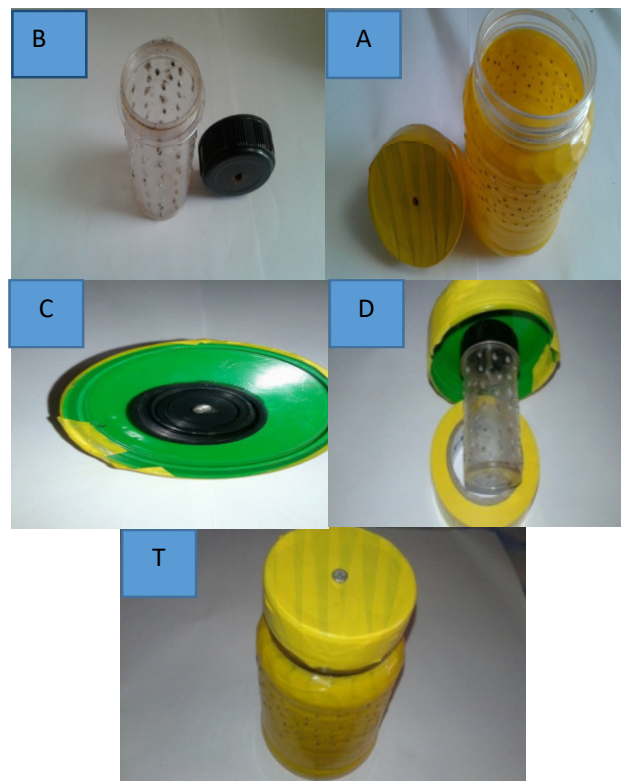


Photo (1) Egg laying pot for peach fruit fly

2.3. artificial diet to the larvae

To feed larvae to study biological aspects. The artificial diet consisting of fine wheat bran was prepared with 500 g, 125 g table sugar, 125 g (Yeast Hydrolysate Enzymatic), 5 g sodium benzoate (C_6H_5COONa), 5 g citric acid and 750 ml water. All the ingredients were mixed in a 2000 ml plastic container and continued mixing the ingredients by a wooden spoon for 15 minutes. The pH of the diet was checked to 3.5-4 by the PH scale by adding a quantity of citric acid (Afia, 2007).

2.4. Study the biological aspects of peach fruit fly at different temperatures in lab

The study was conducted at the laboratory of insects at the Faculty of Agriculture, University of Maysan at temperatures of 20, 25, 30 ± 2 ° C and relative humidity (60-70%). The Memmert incubator was used with 40 W white light bulb with a wavelength of 250-700 nm, For light duration 14 hours light and 10 hours of darkness (Papadopoulos, 1998), the lighting period is set by a timer (Hager). For obtaining relative humidity 60-70% inside the incubator, A glass plate (15 cm diameter and a 2 cm Height) was used by filling it with water as needed, To ensure that the temperature and humidity controlled within the incubator were monitored daily using a thermohygrometer. To study biological aspects and obtain fresh eggs (less than 24 hours), pots designed to lay eggs were placed inside breeding cages, and after fresh eggs were obtained, biological aspects were studied as follows.

2.4.1. Eggs incubation period

Prepared 15 a glass dish of 9 cm diameter (5 dishes representing 5 replicates per temperature). Placed a layer of larvae food in the base of each dish and then placed a black filter paper over the food after moistening with distilled water. Then transfer 10 fresh eggs by a small brush under optical microscope (Pro.Way and 20 X) for each of the five dishes. Placed the dishes in the incubator according to the temperature. The eggs were examined and monitored daily under the microscope, and the date of hatching was observed. The egg incubation period was determined from the date of its incubation until hatching and the percentage of hatching was determined based on the number of eggs and the total number of eggs.

2.4.2. Larval duration

Prepared 15 a glass dish of 9 cm diameter (5 dishes representing 5 replicates per temperature). Placed a layer of larvae food in the base of each dish. Transfer 10 early larvae carefully with a soft brush after checking with microscope (Pro.Way 20 X) to each glass dish. Each dish including larval was moved to plastic container 20 x 10 x 10 cm. Cover the top of the container with a gauze cloth fixed by a rubber band and placed in the incubator according to the temperature. The larval stage was calculated from the date of the first hatch to the egg until it became pupae.

2.4.3. Pupal duration

Prepared 15 a plastic container 20 x 10 x 10 cm (5 container representing 5 replicates per temperature). A layer of sterilized soil was placed at the base thickness of 2 cm, and 10 larvae were transferred to each container. The plastic containers are covered with a gauze cloth and tied with a rubber band. Placed the containers in the incubator according to the temperature and the observation and checking was conducted daily. The duration of pupae was calculate based on start of pupation until adults emerged. The percentage of emerging was calculate based on number of adults to the total number of pupae, and the sex ratio was calculated.

2.4.4. The adult stage

Prepared 15 plastic containers 20 x 10 x 10 cm (5 containers representing 5 replicates per temperature) were prepared. Each container was provided with a 4 cm glass lid containing one part (enzyme hydrozylite yeast) with three parts of sugar and a glass dish containing water, and the plastic container was equipped with a egg laying pot. A pair of adult emerging was provide to each container, which was covered from the top by a muslin cloth and fastened with a rubber band. Observing activity daily , the mating behavior, and eggs laying until laying first group of eggs for each replicate.

The egg laying time was determined from the start of adult emergence to the time of the first set of eggs, and the egg laying time was calculated from the time the first egg was placed to the last egg laying time for each repeat, The egg laying time and the total number of eggs were determined for each replicate, and the examination continued until the normal death of the adult. The age of adults from adult emerged to natural death was calculated at each temperature at which the study was conducted

2.4.5 Virginal Reproductive in lab

As in the previous paragraph, 15 plastic containers are set up with egg-laying pot , water, and food . One insect was transferred to each container, covered with gauze, and placed in the incubator on a temperature basis. The eggs were monitored and counted daily until egg laying stopped. In order to ensure the hatching of non-fertilized eggs, all the treatments were carried out as in (egg incubation period).

2.5. Field studies

2.5.1. Study the life cycle of the insect in field conditions

To study the life aspects of the insect, six wooden cages of 15 x 20 x 20 cm were used, covered with muslin cloth from all sides. One side has a 10 cm circular opening surrounded by a cylindrical muslin cloth. The base of the cage is made of wood, two of insects (male and female), not older than 24 hours were moved for each cage. These cages were placed in the home garden to simulate the field conditions, and the cages were observed a daily, each cage was provided with a 9 cm glass dish containing one part (enzyme hydrozylite yeast) with three parts of sugar and a glass dish containing water , .

The study was conducted on the first week of October, And It lasted until the last week of December, three cages were isolated to study the life aspects of the insect and the other three cages which the fruits were taken from it

and anatomy the fruits to calculate the number of eggs continuously until laying last egg. All life studies were carried out as in the laboratory study, except incubation of eggs and larvae, which was prepared through the preparation of three cages with the same cage specifications described in the previous paragraph. Fruits are examined and monitored until the larvae exit. Egg and larval incubation time is calculated on the basis of egg laying time until the larvae exit the fruit. Continue the process of inspection and monitoring daily for the three cages until the larvae emerge through a hole in the fruit. The date of exit was recorded and calculated the duration of incubation of eggs and larval was calculated by duration from eggs laying until the larvae exit of the fruit.

2.5. 2. Virginal reproduction

To realization the possibility of laying eggs, Virginal reproduction in field conditions in the absence of males. Prepare 6 cages with the same specifications as previously described cages and mandarin fruit on the base of each crate. The female was moved from the insects emerging to each cage (no more than 24 hours), food and water were prepared as in the preceding paragraphs in each cage. Three cages were taken and monitored daily to calculate the number of eggs involved. Fruits were replaced daily, leaving the other three cages to study the biological aspects of insects. The duration was determined before eggs were laid and eggs were laid after egg laying. The study was conducted according to the complete-randomized design (CRD). The results of the study were statistically analyzed by using the variance analysis table and compared to the averages, using the least significant difference L.S.D at the probability level of 0.05 (Al-Sahuki and Waheib, 1990). The SAS 2010 was used for statistical calculations.

3. Results and Discussion

Results in Table 1 indicated the presence of a significant statistical differences between the period for eggs and larvae was significantly affected by temperature. Table (1) showed that the shortest duration was 8.7 days at $30 \pm 2^\circ \text{C}$ while 11.5 days and 17.8 days at 25° and $20 \pm 2^\circ \text{C}$. respectively, and the longest period in the field conditions was 19 days when the minimum temperature ranges were $5.6 - 15^\circ \text{C}$ and the maximum $20.3 - 29^\circ \text{C}$ and relative humidity 17 - 84%. The results also showed a significant statistical differences for the duration of the pupa at temperature of $30 \pm 2^\circ \text{C}$ and the shortest time was 8.2 days at $30 \pm 2^\circ \text{C}$ and 13.2 days at $25 \pm 2^\circ \text{C}$. The longest duration was 19.3 days at $20 \pm 2^\circ \text{C}$. while in the field conditions was 13.6 days, while the results showed significant differences for the pre-egg period. The lowest period was 10.8 days at $30 \pm 2^\circ \text{C}$ and 23.9 days at $20 \pm 2^\circ \text{C}$ and 16.4 days at $25 \pm 2^\circ \text{C}$. While in the field conditions was 22.3 days. The results showed that the shortest egg laying time was 26.4 days at $30 \pm 2^\circ \text{C}$, and the longest 43.6 days at $20 \pm 2^\circ \text{C}$. While 29 days in field conditions, Life cycle duration was 16.9 days at $30 \pm 2^\circ \text{C}$ and the longest life cycle was 37.1 days at $20 \pm 2^\circ \text{C}$ while 24.7 days was $25 \pm 2^\circ \text{C}$ and in field conditions was 32.6 days with a significant difference.

Table (1) the different biological aspects of the peach fruit fly

| Temperature | Duration of egg incubation (day) | Duration of larval stage (day) | Duration of incubation of eggs and larvae (day) | The duration of pupae stage (day) | Pre-egg laying time (day) | Egg laying time (day) | Duration of life cycle |
|--|----------------------------------|--------------------------------|---|-----------------------------------|---------------------------|-----------------------|------------------------|
| $20 \pm 2^\circ \text{C}$ | 4.3 | 13.5 | 17.8 | 19.3 | 23.9 | 43.6 | 37.1 |
| $25 \pm 2^\circ \text{C}$ | 3.2 | 8.3 | 11.5 | 13.2 | 16.4 | 32.8 | 24.7 |
| $30 \pm 2^\circ \text{C}$ | 2.4 | 6.3 | 8.7 | 8.2 | 10.8 | 26.4 | 16.9 |
| Field conditions | – | – | 19 | 13.6 | 22.3 | 29 | 32.6 |
| the least significant difference at 0.05 | 0.39 | 0.70 | 0.89 | 1.38 | 1.33 | 3.5 | 1.58 |

Results showed that the highest emergence of adults rate was achieved at 25°C , $30 \pm 2^\circ \text{C}$, 86% and 68% at $20 \pm 2^\circ \text{C}$ and that the lowest incidence in field conditions was 63% with statistical difference and the lowest egg-laying rate was recorded in the field conditions. It reached 70.8 eggs/female while the highest egg-laying rate

was recorded at 30 and $25 \pm 2^\circ \text{C}$ and it was 218,215.2 eggs / female and without significant statistical difference between the two degrees while 171.2 eggs /female at $20 \pm 2^\circ \text{C}$. While the shortest post- egg laying-period was 4.2 and 4.8 days at 30 and $25 \pm 2^\circ \text{C}$ respectively without significant difference between the two degrees. The longest duration was 9.4 days at $20 \pm 2^\circ \text{C}$ and 5.3 days in the field conditions. The highest egg hatch rate was 80% at $25 \pm 2^\circ \text{C}$ and the lowest hatch rate was 64% at $20 \pm 2^\circ \text{C}$, hatching ratio was 78% at $30 \pm 2^\circ \text{C}$. On the other hand; hatching was not recorded in field conditions because of the difficulty of calculating the number of eggs and the percentage of hatching in the fruit. The results of the study showed that the male / female ratio of peach fruit fly eggs at $20 \pm 2^\circ \text{C}$, $25 \pm 30^\circ \text{C}$ and field conditions were 0.54: 1, 0.79: 1, and 0.58: 1 respectively.

Table (2) some different biological aspects of the peach fruit fly

| Temperature | Emergence % | Eggs number (egg/female) | Post-egg laying period (day) | Percentage egg hatching % | Percentage Sex ratio |
|--|-------------|--------------------------|------------------------------|---------------------------|----------------------|
| $20 \pm 2^\circ \text{C}$ | 68 | 171.2 | 9.4 | 64 | 0.54:1 |
| $25 \pm 2^\circ \text{C}$ | 86 | 215.2 | 4.8 | 80 | 0.79:1 |
| $30 \pm 2^\circ \text{C}$ | 86 | 218 | 4.2 | 78 | 0.79:1 |
| Field conditions | 63 | 70.8 | 5.3 | – | 0.58:1 |
| the least significant difference at 0.05 | 0.82 | 27.8 | 1.14 | 1.37 | |

The study also showed that females isolated from males managed to lay eggs, but the eggs were not vaccinated and did not succeed in hatching (Table 3), which confirms the absence of the phenomenon Virginal reproduction in the peach fruit fly. The statistical analysis proved that there is no statistical difference between the number of eggs at the level The temperature of 25 and $30 \pm 2^\circ \text{C}$, which was 81.4 and 83.2 eggs/female, respectively, while 65.4 eggs / female at $20 \pm 2^\circ \text{C}$ and 41 eggs / female at field conditions and a significant statistical difference. The study also found that the age of the female is inversely proportional to temperatures. The longest age of the female was 80.4 days at $20 \pm 2^\circ \text{C}$, while the shortest age for females was 44.4 days M $30 \pm 2^\circ \text{C}$ was at 58.8 ± 2 m 25° while the 62.3 day in the field conditions and the significant difference statistically.

Table (3) the number of eggs, the percentage of eggs hatching and the length of females which Virginal reproduction.

| Temperature | Number of Eggs laying / Female | Hatching (%) | Duration of Female Age |
|--|--------------------------------|--------------|------------------------|
| $20 \pm 2^\circ \text{C}$ | 65.4 | 0 | 80.4 |
| $25 \pm 2^\circ \text{C}$ | 81.4 | 0 | 58.8 |
| $30 \pm 2^\circ \text{C}$ | 83.2 | 0 | 44.4 |
| Field conditions | 41 | 0 | 62.3 |
| the least significant difference at 0.05 | 12.36 | | 7.89 |

It is obvious from table (1, 2) that all the stages life cycle of the peach fruit fly is shortened as the temperature rises. The shortness of these periods may be due to the large role of temperature in influencing physiological processes, the evolution of embryos within eggs, the growth of other roles and the speed of metabolic processes. In this context, Chapman (1988) pointed out that the period of growth and development fetus decreases with increasing temperatures, this is confirmed by Mohamd (2009). The period of incubation of the eggs and the development of the peach fruit fly stages decreases with temperatures high from 20 to 30°C and the longest incubation period 20°C is 4.66 days and Younes (2010) noted that the decreased development of embryonic and development of larvae, pupa with increasing temperatures from 20 to 35°C . It was also observed that adult emerged, egg-laying rate, egg hatch rate and male sex ratio were correlated with Positive relationship with temperature high from $20 \pm 2^\circ \text{C}$ to 25°C and 30 ± 2 , and emerging adults die after emergence in the absence

of food for more than two days and the results are consistent with what Al-Joboory (2009) found that the Mediterranean fruit fly *Ceratitias capitata* dies after emergence in the case of non-feeding for 2-3 days, the reason of shorten the post-egg duration at $\pm 30^{\circ}\text{C}$ to depletion stored nutrients and stress. The percentage of females was generally higher than that of males at all temperatures used in the study. Mohamd (2009) indicated that the temperature of $25 \pm 2^{\circ}\text{C}$ was the ideal for the fruit fly Peach, Afia (2007) confirmed that the sexual ratio of males increases with higher temperatures. The results table (3) showed that sexual reproduction is the main way to produce fertile generations in the peach fruit fly, in spite of the female is lay the eggs without mating, But the eggs do not hatch whatever different temperatures. Chapman et al (1998) confirmed that some species of insects produce eggs that are not fertilized but do not hatch. It can also be concluded that the virgin female age is longer than the age of mated female and according to different temperatures. This may be due to the efforts exerted by the mated female to lay larger numbers of eggs and the depletion of larger amounts of food associated with it.

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