



Research Article

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Biological and Reproductive Characteristics of the Mediterranean Fruit Fly, *Ceratitis capitata* (Dip.: Tephritidae), on Six Host Plants Under Vitro Conditions



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Abstract

Mediterranean fruit fly, (Wiedemann) *Ceratitis capitata*, is one of the most problematic pests of fruit trees in the world, which has been reported in recent years in the fruit gardens of northern Iran. In this research, some of the important biological and reproductive characteristics of this fly including the average daily egg in two selective and non-selective tests, the length of larval and pupal periods, the percentage of hatching eggs, the percentage of total insect excretion and sex ratio on six different plant hosts persimmons, Clementin mandarin, Valencia oranges, Golden apple smoothie, Red apples, and peaches) were measured in laboratory conditions. Among the studied hosts, peach contributed to the highest average egg density of 4.74 eggs per individual fly/day and a hatching percentage of (62.79%). Moreover, it exhibited short duration of larval stages (4.10 days) and pupation (7.76 days), the most favorable host for Mediterranean flies was determined. In contrast, Valencia oranges were identified as the most undesirable host because of the low average egg density and egg hatching percentage (zero and 67.49%), respectively. Also, the yellow and red apple fruits also indicated low average daily egg laying (respectively, 0/56 and 1/15 eggs per individual fly/day) and the length of the larval stage (14.84 and 24.08 days) were established as low-quality food hosts for this fly. Due to the high sensitivity of peach fruits to this pest, it is recommended to exercise prudence in the construction of new peach orchards, especially mixed with citrus.

Keywords: Host preference; Host plant; Nutrition and fruit fly; *Ceratitis capitata*

Introduction

The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) is a polyphagous pest. So far, more than 400 plant species have been reported as its host (2012 USDA), of which the most important are citrus, nutrient fruits such as peaches, grainy fruits such as apples and other ones such as persimmons, figs and even the fruits of some gourds [1]. In some countries, commercial production is riddled by some serious challenges and even impossibility because of the Mediterranean fruit fly damage. In California, this fly accounts for an annual financial loss of about \$1.1 billion [2]. The damage caused by this pest in Europe was reported at 68 and 71% respectively in tangerines (*Citrus reticulata* Blanco and Japanese persimmons (*Diospyros kaki* Thunb) respectively. Currently, in many parts of the world, the use of insecticides is the most effective alternative in controlling the Mediterranean fruit fly and the availability of appropriate diets for the cultivation of large amounts of larvae and insects is one of the main pillars of this way [3]. The quality and quantity of food are very important in feeding the larvae of the Mediterranean fruit fly, because in addition

to providing the energy necessary for the survival and appearance of the larvae, they can also be used in the body during their inactivity period [4,5]. The nutritional effects of plant varieties on the biological and reproductive characteristics of this group of flies have been reported [6-9]. By assessing the nutritional value of different hosts for larvae and adult insects, the Mediterranean fruit fly showed that the highest percentage of insect excretion and egg numbers were observed in mango fruits, while lemon and apple fruits did not have any complete outflow flies failed. The study of viability and the appearance of immature larvae of the Mediterranean fruit fly on citrus varieties showed that the fruit variety and different parts of the fruit had a significant effect on the larval function, but the effect of pepper (*Capiscum annum*) was particularly lower and on the eggs, no effect was indicated [9]. Studies by [2] showed that the Kiwi fruit, in comparison to the nectarine fruit, was less favorable for the Mediterranean fruit fly [2]. The effect of different species and varieties of host plants on the demographic parameters of the Mediterranean fruit fly, including

the inherent rate of population growth, has also been reported Papanastasiou et al. [10]. The Mediterranean fruit fly was introduced to Iran through imports of fresh fruits from 1973 to 1979 and quickly settled in the country's fruit producing regions [11]. The activity of this pest in recent years has caused severe damage in Tehran-Varamin Research Farm University of Varamin is located in the southern region of Tehran City, Geographically, this training farm is located at 51 degrees and 38 minutes north latitude and 35 degrees and 19 minutes east longitude with a height of 920 meters above sea level. [12,13]. Despite the economic importance of Mediterranean fruit fly in Iran, so far, limited studies have been conducted on the effect of the type of diet on reproduction and its reproduction [12, 14,15].

Therefore, this study was conducted to investigate the effect of feeding the larvae of this fly on several species of important and common hosts in Tehran-Varamin province on its characteristics and its reproductive characteristics in vitro. Knowing the nutritional quality of different host plants, in addition to improving the efficiency of the pest management program, can help predict the trend and pattern of infestation in Tehran-Varamin province.

Materials and Methods

The place and time of the research

This study was conducted during the period of 2017-2018 at the Plant Protection Research Institute of Peoples' Friendship University of Russia (PFUR).

Preparation of primary population

The primary population of the fresh fruit flies was made up of four pairs of adult insects from a laboratory belonging to the department of plant protection, PFUR in Russia (Figure 1). In this lab, the population was raised for five generations on an artificial diet (a diet based on wheat bran for larvae and a diet based on hydrolyzed protein for full insects).



Figure 1: *Ceratitis capitata* (Photo taken by the author of the article in the laboratory).

Purification and propagation of the population

Due to the low number of the primary population and also to create a colony with identical nutritional conditions, the initial population of the flies for three generations was grown on *Prunus persica* L. and then for two generations on artificial diet (five generations in total). In order to cultivate peach fruits, the primary population was covered in wooden cages of 50×50×50cm, with

their ceilings and walls wrapped with wire holes 0.2mm in diameter (approximately 50 meshes). In order to access the interior of the cages in one side, a hole in the diameter of 10cm was created and a piece of white shirt in the form of a sleeve was sewn and inserted into it. The cages were placed in a growing room of 2 × 3m with a temperature of 25±2 °C, relative humidity of 65 ± 10 °C, an optimal period of 12 hours of light and 12 hours of darkness. Every two to three days, three to four peach fruits were placed on the floor of the cage for the laying of female flies in order to feed adult insects in the cages, a diet based on hydrolyzed protein was used [16,17].

The infested fruit was gradually removed from the cages and placed in plastic pots with a diameter of 22cm and a height of 15cm with holes of 3cm diameter on the floor. Pots containing infested fruits were blocked using a Lace fabric and the pots were placed on plastic containers containing a mixture of fine soil and sawdust d (at a thickness of 3cm and as a bedding for saplings). The pupas were placed in plastic containers containing a mixture of fine soil and sawdust and transferred to new cages to remove insects from them. Due to the lack of access to peach fruits all year round and after the arrival of the population density of the flies at a desirable level, it was used to continue the development of artificial diets proposed by Martínez-Ramírez et al. [16]. Artificial food was used to feed full flies in liquid form, and each unit contained warm gr of liquid hydrolyzed protein, three gr of sugar and 25ml of distilled water. Each artificial larvae, unit was completely and sufficiently sustained by 100g of wheat bran, 25g of sugar, 25g of powdered beer yeast from (Health Aid Company of UK), one gram of hydrochloric acid, one gram of sodium benzoate and 210ml of distilled water.

Biological and reproductive characteristics

Daily egg laying average: Two non-selective and selective tests were used to measure the average daily egg. In the non-selective test, the persimmon fruits (*Diospyros sp.*), Citrus reticulata blanco and Valencia oranges (*Citrus cinensis* (L)) were used as experimental treatments alone and without other fruits. For this purpose, approximately 20 pairs of full-day flies fed on artificial nutrition during the larval period were dispensed with a wooden cage with dimensions of 25×25×25cm, and the protein mixture was hydrolyzed, and water fed to them. Given that the average period before mating of this swarm was reported to be at least four days, after four days the flies were placed inside the cages. A piece of the camellia fruit was cut from it within each cage given. After 24 hours, all fruits were removed from the cages and fresh fruits were replaced. This action lasted for 10 days. Having been removed from the egg cages, the fruits were carefully checked under the stereomicroscope, and the eggs were marked on them.

In the next step, the marked sites were split using insect needles and the number of eggs laid in each of them was counted. By dividing the number of eggs laid per day by the number of flies in the cage, the average daily egg was calculated on different fruits. In the selective test, from apple fruits, *Malus domestic* (Borkh)

(Golden smoothie and Red) and Peach (sixty-day cultivar) were used as experimental treatments. At first, 20 pairs of swarms that had reached the laying period were released into the wooden log cabin. Then, all three fruits were placed in the cage simultaneously and together. After 12 hours, the fruits were removed from the cage and healthy fruits were replaced. Then, the number of eggs laid in them was counted as before. By dividing the total number of eggs counted on each fruit with the total number of flies in the cage (including the number of dead flies per day), the average daily egg was calculated on each fruit.

Hatching eggs: According to the Hussain [18] method, Clementin mandarin, apple (Golden smoothie and Red) and peaches (sixty-day) cultivars put in the wooden cages that were pre-purified for Mediterranean fruit (maximum 24 hours) during their growing season [18]. After 24 hours, the fruits were removed from the cage and the laying sites were marked under a stereomicroscope. The fruits were then placed in the germinator ($25 \pm 2^\circ\text{C}$, $70 \pm 5\%$, 12 hours' light and 12 hours' darkness). Given that the length of the embryonic period of the flies was reported to be 1.5 to three days and given the maximum length of time that it was possible to store split fruits inside the germinator, tangerines, persimmon, peaches were removed after four days, and Valencia orange fruits, yellow apples and red apples were removed from the germinator after six days [19]. All the laying sites were split over them and the number of hatching eggs remained (based on the number of egg shells) and percentage of hatching eggs were calculated. Due to the low level of normal egg laying in the Valencia oranges, a sharp knife was used to cut the skin in semi-circular orientation and a certain number of eggs were placed under the skin and on the fruit. Then, the cut was restored to its original location and tightened with tape.

Larval and pupae age: After determining the percentage of hatched eggs, each contaminated fruit was separately packaged in a plastic container with a diameter of 10cm and a height of 7cm and a layer of a centimeter mixture of fine soil and sawdust and multi-layer newspaper (as a substrate of pupae). The craters of the dishes were covered with lace fabric and then turned into germinator. The dishes were regularly visited until the pupae were formed. The time interval between egg hatching on each fruit and the formation of saplings was determined as the length of the period and the larval aspect. In order to determine the length of the pupae period, the soil in containers that had infested fruits was observed on a daily basis until the key removal of the larvae from the fruits and their transfer into the soil to form pupas. The pupas were collected per day depending on the type of fruit and placed in 8cm diameter petri dishes that were covered with a centimeter layer of mixture containing fine soil and sawdust. Then, petri dishes containing pupas were kept until the insects were completely embedded in the germinator. The time interval between the formation of pupae to the complete extinction of insects was considered as the duration of the pupal period.

Percentage of insect outflow and the proportion of female insects: In order to calculate the total insect outflow rate and sex

ratio of individuals, the number of pupae per fruit and the number of insects removed from them were counted on a daily basis, and the sex of all the flies was determined based on the presence or absence of egg yolks.

Statistical analysis

All experiments were conducted in a completely randomized design. The treatments in this study included persimmon, Tangerine, Valencia orange, yellow apples of Golden smoothie, red apples and peaches (sixty-day cultivar). Due to technical problems (for example, lack of sufficient swarms of flies and non-simultaneous possession of all fruits by reason of different growing seasons, some characteristics were not measured on some fruits. The number of replicates varied according to the type of character; the average daily egg, hatching percentage, total insect emergence percentage and female percentage in 10 replicates and length of larval and pupal periods in 25 replicates were measured. The data were analyzed using SAS (SAS 1999, Institute) software and the meanings were compared using Duncan's multi-domain test and at 5% probability level. Excel 2007 software was used to draw charts.

Results

Daily egg laying average

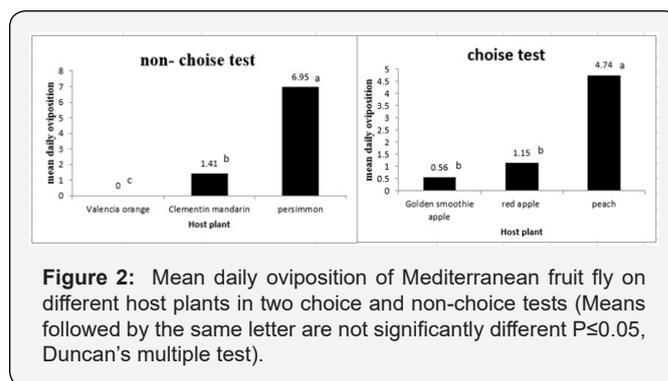


Figure 2: Mean daily oviposition of Mediterranean fruit fly on different host plants in two choice and non-choice tests (Means followed by the same letter are not significantly different $P \leq 0.05$, Duncan's multiple test).

Analysis of variance showed that in both selective and non-selective tests, the host plant type at the level of probability influenced the percentage of average daily laying of female flies ($P \leq 0.05$). Based on comparison of the averages in the non-selective test (Figure 2), the highest and lowest mean of eggs laid were observed on persimmon fruits (6.95 ± 0.68 eggs per female per day) and Valencia oranges (0 eggs per female per day). The average daily egg density of Clementin mandarin was lower than persimmons but more than Valencia oranges. In the selective test (Figure 2), the mean daily egg laying of female flies on peach fruits was significantly higher than apple fruits (Lebanese yellow and Red varieties), but there was no significant difference between the two apple cultivars.

Period of pupal development

The analysis of variance showed that the plant host type had a significant effect on the length of the larval period ($P \leq 0.05$, and pupae, $P \leq 0.05$.) Mediterranean fly has a significant impact. Lar-

val period and larval stage varied from 8.92 ± 0.33 days to 24.08 ± 1.67 days, respectively, on Valencia orange and red apple fruits.

The lowest period of pupas (7.76 ± 0.3 days) was in peaches and the highest in persimmons fruit (13.84 ± 0.59 days) (Table 1).

Table 1: Means (\pm SE) of biological parameters of Mediterranean fruit fly on different host plants.

Host Plants	Larval Developmental Time (days)	Pupal Developmental Time (days)	Egg Hatching Percent	Adult Emergence Percent	% Females
Valencia orange	8.92 \pm 0.33d	12.04 \pm 0.32b	67.49 \pm 10.72b	98.55 \pm 0.88a	59.35 \pm 3.01a
Peach	10.40 \pm 0.46cd	7.76 \pm 0.30d	79.62 \pm 9.44ab	82.82 \pm 4.25a	57.09 \pm 2.7a
Clementin mandarin	10.68 \pm 0.53cd	12.12 \pm 0.29b	93.65 \pm 5.03a	95.97 \pm 2.01a	52.85 \pm 2.07a
Persimmon	12.04 \pm 0.94c	13.84 \pm 0.59a	68.48 \pm 4.62b	-	50.00 \pm 7.7a
Golden apple smoothie	14.84 \pm 0.81b	9.72 \pm 0.46c	87.82 \pm 2.84a	83.72 \pm 5.7a	52.65 \pm 6.9a
Red apple	24.08 \pm 1.67a	8.28 \pm 0.81d	90.3 \pm 1.67a	81.08 \pm 8.6a	43.25 \pm 10.05a

* Means within columns followed by the same letter are not significantly different ($P < 0.05$, Duncan's multiple test).

Hatched eggs

Analysis of variance showed that the effect of host plant on hatching percentage was significant at level of probability ($P \leq 0.05$) Based on the results of the comparison of the averages (Table 1), the highest number of eggs after feeding the larvae of the hatching Clementin mandarin fruit ($93.65 \pm 5.03\%$), while the lowest egg hatching ($67.49 \pm 10.72\%$) was observed after larvae feeding from Valencia orange fruits.

Percentage of insect outflow and proportion of female insects

Mean variance analysis showed that plant host type had no significant effect on the percentage of emergence of insects and the ratio of female subjects ($P > 0.05$). Based on the comparison of the means, the average emergence rate of the adult insects was from $81.08 \pm 8.6\%$ in the red apple to $98.55 \pm 0.88\%$ in the case of Valencia oranges and the mean the proportion of females varied from $43.25 \pm 10.05\%$ in red apples to $59.35 \pm 3.01\%$ in Valencia orange (Table 1).

Discussion

The results of this study showed that among the biological and reproductive characteristics of the Mediterranean fruit fly, the average daily egg laying, larval stage and pupae, and the percentage of hatched eggs were affected by the type of fruit. However, the percentage of total insect outflow and the ratio of individuals were not influenced. The material did not differ significantly in different fruits. The effect of fruit type on the biological and reproductive characteristics of Mediterranean fruit fly [9,16,20] and other fruit flies [21]. These characteristics are influenced by various traits of the different fruits, such as differences in the thickness of the fruit skin [9,22], fruit color [23], fruit size [24], relative humidity within the fruit [25], fruit quality for larvae [25], and the density of the essential oils in the fruit juice [4,23]. According to the results of various researchers in this regard, the difference in the values of biological properties and reproductive production on different fruits in the present study may be due to some of the above-men-

tioned factors, which confirms that this particular aspect requires further research.

Effect of fruit type on mediterranean fruit flies

The findings of this research in non-selective test indicated that the number of female flies was low on Valencia orange and Clementin mandarin fruit compared to persimmons fruit. This result was consistent with Chang and Follett's findings [2,4] and [26,27]. Szyniszewska et al. [28] and Tanga et al. [29], in justifying the low mean of female flies on orange fruits and citrus fruits (*C. limon* L.), showed that between the regeneration of female flies and endocrine densities there was a negative correlation between the secretion and the amount of essential oils in the fruit of the citrus fruit. For this reason, the researchers attributed the non-infestation of these fruits by the Mediterranean fruit flies to the presence of essential oils in their skin. The resistance of different varieties of citrus has been reported in different Mediterranean fruit flies. Papanastasiou et al. [10] showed that the emergence of Mediterranean fruit fly on some citrus varieties such as orange (*C. aurantium* L.) was at a relatively high level [10,23]. The researchers have shown that the lemons are safe against the attack, and the orange is largely resistant. Also, the results of the present study in the selective test showed that female flies for oviposition preferred peach fruits to red and yellow apple. So far, the comparative study of the preference of peaches and apple fruits for Mediterranean flies has not been reported.

But Mahmoud et al. [30] and El-Hawagry [31] Based on the number of pupas in the fruit and the percentage of insect excretion, they concluded that the apple fruits are more resistant to Mediterranean flies than orange fruits [30,31]. Joachim-Bravo et al. [32] also, based on the percentage of total insects' emergence and duration, the Mediterranean fruit flies preferred papaya fruits (*Carica papaya* L.) more than apple fruits [32]. In contrast, [22], Contrary to our research results, the number of eggs laid by the Mediterranean fruit fly on apple fruits (cultivars Golden smoothie) is much higher than Valencia oranges and Clementin mandarin [22].

Effect of fruit type on egg hatching

The results of this study showed that the percentage of hatched eggs on Valencia orange or persimmons fruits was lower than those of red and yellow apple fruits, peaches and Clementin mandarin. [23,26] studying the effect of different species and varieties of citrus on hatching percentage of Mediterranean flies, showed that, despite the difference in skin thickness, acidity, pH, in citrus species and cultivars, the percentage of hatched eggs in each other did not differ significantly [23,26]. Therefore, in order to justify the low percentage of egg hatching on different species and varieties of citrus, the role of other physical and chemical characteristics of the fruit should be investigated. Despite the low average egg content in Clementin mandarin fruits, a high percentage of eggs were hatched, while in Valencia orange fruits, in addition to lower average egg laying, the percentage of hatching eggs was also very low.

This may be due to the different physical structure of the skin and the chemical nature of the essential oils contained in Clementin mandarin fruits compared to the Valencia orange. The basis of the total average egg yield and the percentage of egg hatching can be that the essential oils contained in the skin of the Clementin mandarin fruit only prevent the laying of female flies on the fruit (the role of antioxidants), but their concentration and their chemical nature to inhibit hatching is not enough within the skin of the fruit. Moreover, the probable second-order metabolic compounds present in the skin of Valencia oranges, play a reverse role and prevent the hatching of eggs. According to the results of this study, the average egg contained in persimmons fruits was significantly higher than those of Valencia orange and Clementin mandarin, but the percentage of hatching eggs in it was much lower than those of Clementin mandarin. In justifying this phenomenon, it can be said that the antioxidants are probably not strong in persimmon fruits, and therefore female flies can easily deposit a large number of eggs within them, but the chemical and physical nature of the fruit prevents a large number of eggs from hatching.

The effect of fruit type on the length of the period and the appearance of larvae and pupa

The results of this study showed that the period of larvae of Mediterranean fruit flies in apple fruits (red and yellow) was considerably longer than other fruits. This corresponds to the results of [33] regarding the longer larval period of Mediterranean fruit fly on apple fruits compared to peach and persimmon fruits. But the results of these researchers were slightly different regarding the length of the pupae period of our research; the results indicated that there was no significant difference between the pupae period in persimmon, apples, and peaches, while our research findings showed no significant differences between peach and apple fruits [33].

Nevertheless, the average of both fruits was significantly lower than persimmons. Given the long larval period on apple fruits, as well as the relative lack of average egg on this host, yellow and

red apple fruits, along with Valencia oranges (with a mean egg setting of zero), was introduced to the most unviable host plants for the Mediterranean fruit fly. Papadopoulou et al. [23] and Fernandes-da-Silva [34], reported the mild and bitterness of the Mesopotamian carp apple fruits as the most important cause of larval mortality and prolonged periods. In the present study, the more rigid mesocarp of apple fruits than other fruits such as peaches, persimmons and citrus fruits, could have contributed to the prolonged larval period [23,34].

The results of this study showed that the Mediterranean flies did not have eggs in Valencia oranges, but the larvae that had been removed from the eggs by hand under the skin of this fruit (artificial contamination), infested the faces of this fruit faster. This reveals that Valencia orange fruits have a high nutritional value for Mediterranean fruit fly larvae. If female flies overcome the barriers to egg laying on fruit (possibly high concentrations of essential oils with secondary metabolic compounds), the possibility of spreading contamination on this host is very high. There are many. Our results also showed that the length of the period of larvae and the appearance of larvae in peach and persimmon fruits was significantly shorter. Due to the high average daily egg laying on these two fruits, they can be introduced as the preferred hosts of these flies.

The impact of the quality of the larval period diet on the length of the period and the appearance of the larvae and pupae of the Mediterranean fly in its bulk on artificial foods has been reported [35,36]. Research by [37], showed that with increasing amounts of protein and sugar components in larvae diet, the duration of their growth and their appearance significantly decreased [37]. Although in the present study, the quantity and quality of the nutrients found in the fruits were not evaluated. Nonetheless, the comparison of the nutritional compounds reported for these fruits suggests a difference between them. For example, the protein content of one hundred gr of apple juice (0.1-0.49g) is much lower compared to orange fruits (0.8-0.9g), Noori et al. [38]. This difference in protein content, as well as the hardness of the mesoporous tissue in apple fruits, may be accountable for prolonged periods and larval forms [13,38].

Conclusion and Suggestion

Among the different fruits in this study, peach seems to be the most suitable host for Mediterranean flies due to the high average egg density and the percentage of hatching eggs and the short duration of larvae and pupae. In contrast, due to the low average egg laying and the percentage of hatching eggs in Valencia orange fruits compared to other hosts, this fruit was reported as the most unfavorable host for this fly. The average daily egg production on persimmon fruits was higher than other hosts, but the percentage of egg hatching within them was relatively low and the period of larvae and pupae in them was long as well. For this reason, this plant can be described as an average quality host for this fly. Yellow and red apple fruits were introduced as low-quality food hosts due to the low daily oviposition and prolonged period of larvae. Con-

sidering the greater sensitivity of peach fruits than other hosts, as well as unpublished reports from plant protection experts in Tehran-Varamin province on the increased damage caused by this pest in citrus and citrus gardens, in the construction of gardens of nucleated trees, including peaches in the province, especially in mixed crops, there should be more stringent measures.

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