

## Determination of Host Preferences of Medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), in the Laboratory

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### Abstract

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is the most important and destructive pest in the world. The aim of this study was to determine host preference and survival of Medfly in different fruit and vegetable cultivars in the laboratory. Medfly was recently established in Çanakkale province, even though it has been known in southern Turkey for many years. Medfly infested peaches were collected to establish a local laboratory population. Ten different hosts were tested in the controlled laboratory conditions. Some biological parameters were observed to find out the preferred and appropriate host for laboratory rearing. The tested fruit weight, the number of oviposition stings, pupal yield and the adult emergence were determined. The results showed that fruit weight and oviposition stings were not related with larval survival or pupal yield of Medfly. Screening of the different host cultivars suggested that red sweet pepper (*Capsicum annuum* L. var. "Kapya") is the most favorable host for Medfly rearing in the laboratory conditions. Therefore, ovipositional preference of Medfly and the success of larval development in different host varieties are crucial for Çanakkale since it is an important city for exporting fruit and vegetables.

**Key words:** Medfly, Mediterranean fruit fly, Çanakkale, oviposition sting

## Akdeniz Meyve Sineği'nin, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), Laboratuvarda Konukçu Tercihinin Belirlenmesi

### Özet

Akdeniz meyve sineği, *Ceratitis capitata* (Wiedemann), dünyada çok önemli zararlar meydana getiren türdür. Çalışmanın amacı, laboratuvarda Akdeniz meyve sineğinin, farklı meyve ve sebze türlerini tercih etme durumunun ve canlılık oranlarının belirlenmesidir. Akdeniz meyve sineği uzun yıllardır Türkiye'nin güneyinde bulunmasına rağmen, Çanakkale ve çevresinde yakın zamanda tespit edilmiştir. Laboratuvar koşullarında 10 farklı konukçu test edildi. Laboratuvarda yetiştirilmesinde tercih edilen ve en uygun konukçunun belirlenmesi için bazı biyolojik parametreler değerlendirildi. Test edilen meyvenin ağırlığı, yumurta izi sayısı, pupa sayısı ve ergin çıkışları belirlendi. Sonuç olarak, meyve ağırlığı ve yumurta izi sayısının Akdeniz meyve sineğinin larva canlılığına ve pupa miktarına bağlı olmadığı tespit edildi. Farklı konukçuların test edilmesi sonucunda, kırmızı tatlı biber (*Capsicum annuum* L. var. "Kapya")'ın laboratuvar koşullarında Akdeniz meyve sineğinin yetiştirilmesinde en fazla tercih edildiği belirlendi. Dolayısıyla, Akdeniz meyve sineğinin yumurta bırakma tercihi, farklı konukçularda larva gelişme başarısı meyve ve sebzelerin ihracatında önemli bir şehir olana Çanakkale için oldukça önemlidir.

**Anahtar kelimeler:** Akdeniz meyve sineği, Çanakkale, yumurta bırakma izi

## Introduction

The Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera:Tephritidae) is one of the most important fruit pest in the world. It is commonly called Medfly, attacking more than 260 different hosts as fruits, vegetables and nuts (Liquidó et al., 1991). It is originally belonging to sub-Saharan Africa and widespread throughout Africa (Gasper et al., 1991; Malacrida et al., 1992). It is an invasive pest and currently well established in the Middle East, Mediterranean countries, Australia, Central and South America, the Hawaiian Islands and the Caribbean (Liquidó et al., 1991). It has an extensive dispersive ability and tolerance to live in wide temperature range (Malacrida et al., 1998; Malacrida et al., 2007). The damage caused by larvae affecting high commercial value productions. There are several management strategies such as monitoring and prevention methods for Medfly infestation reached billions of dollars each year (Liquidó et al., 1991; Erkerlin, 2005). Medfly is an EPPO A2 quarantine pest (OEPP/EPPO) and is also extremely important throughout the world's quarantine restrictions (CPPC, NAPPO, APPPC) (Christenson and Foote, 1960; Anonymus, 2017).

Medfly is a well-known and studied tephritid fruit fly species throughout the world. There are numerous studies on the biology (Carey et al., 2008), behavior (Clausen, 1978), biological control (Cunningham, 1989) physiology and parapheromones (Duyck and Quilici, 2002), survival (Fletcher, 1989), ecology (Cunningham, 1989), control (Gilmore, 1989 and Gasparich et al., 1997), genetic structures and whole genome sequence (Gasperi et al., 2002; Costa et al., 2011; Papnicolaou et al., 2016). These studies have provided an extended understanding of the biology, pheromone trapping, distribution, host species and genetic aspects of the Medfly.

Medfly was an endemic pest in Turkey, established in Mediterranean and Aegean coasts of Turkey for many years ago and mainly fed on Citrus. But now, Medfly introduced as small population in early September 2016 in Çanakkale, northwestern Turkey.

Çanakkale is an agricultural city, growing mainly wheat, barley, rye and oats. It has also heterogeneous fruit orchards, producing and exporting plum, peach, apricot, cherry, pear, apple, fig, grape, olive and persimmon. Most of the produces will be negatively affected by Medfly invasion because of the wide host range.

It is known that host preferences of Medfly vary in the world. This may be related with its ability to adapt and move into new areas easily (Costa et al., 2011). The pest's biology and

oviposition behavior are important factors to understand its host preferences (Aluja and Mangan, 2008). The potential host is determined as host but not yet infested in the nature; however it can be infested, completed their development and reproduced in controlled laboratory conditions (Genc, 2016).

The aim of the present work was to evaluate the laboratory infestations of ten economically mature fruits and vegetables. The different hosts were screened to understand the development, survival and oviposition behavior of Medfly.

## Materials and Methods

Field-infested peaches (*Prunus persica* L. and figs (*Ficus carica* L.) were collected in September 2016 in Çanakkale province, Turkey. Emerged flies were reared in the laboratory for several generations on peaches and figs before used in the experiments. We followed up rearing protocols used for other tephritids in the laboratory (Edwards and Wratten, 1980; Genc and Nation, 2008). Newly emerged adults (100♀:100♂) were put in mesh screen cages (30X30X30 cm) provided with adult food both as cube sugar alone and standard diet (a mixture of yeast hydrolysate and granulated sugar at 1:3 ratio). Slices of Chinese pear or Nashi pear cultivar (*Pyrus pyrifolia* Nashi.) was also placed in cages as adult food just because of Medfly liked to suck sweet and juicy Nashi Pear. Water was supplied in a cup with dental wicks (Fig. 1).

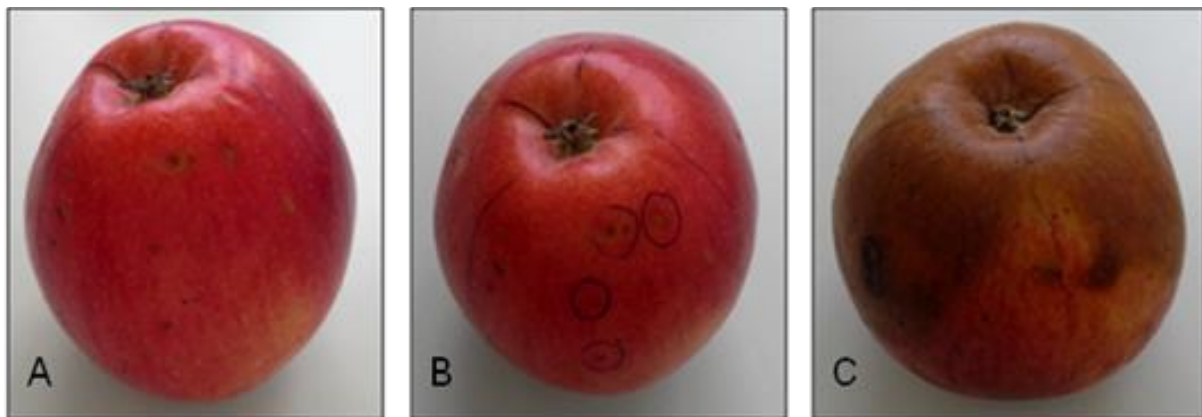
In order to determine the infestation status of the different hosts on Medfly, several commonly produced fruits and vegetables were tested in the laboratory. The tested fruits were Satsuma mandarine (*Citrus unshiu* Marc.), Murcott mandarine (*Citrus reticulata* cv. "Murcott"), pear (*Prunus communis* L.), Nashi pear (*Pyrus pyrifolia* Nashi.), pear Deveci cultivar (*Prunus communis* Deveci), plum (*Prunus domestica* L.), persimmon (*Diospyros kaki* L.), apple (*Malus domestica* cv. Golden delicious), apple pink lady cultivar (*Malus domestica* cv. "Pink Lady"), Orange (*Citrus sinensis* L.), medlar (*Mespilus germanica* L.), Green Bell Pepper (*Capsicum annuum* L), sweet pepper kapyra cultivar (*Capsicum annuum* L. var. "Kapyra") and a cherry tomato (*Solanum lycopersicum* var. *cerasiforme*). All tested hosts were economically important and mostly produced in Çanakkale. All fruits and vegetables (n=3) were used in this study were fully mature and purchased from the local market. The tested hosts were washed thoroughly with tap water and air dried and then weighted before placed in the experimental cages. The hosts were individually kept in adult cages for 2 days for

oviposition (Fig. 2). Medfly females deposit eggs in groups inside the apple, *Malus domestica* cv. “Pink Lady” (Fig. 2(a)), ovipositions stings were marked

for counting (Fig. 2(b)), and kept infested fruit for larval development (Fig. 2(c)) in the laboratory. All hosts were tested as above.



**Figure 1.** An inside view of Medfly laboratory rearing cage



**Figure 2.** The infested *Malus domestica* cv. “Pink Lady” (a) Apple was divided by marker pen (b) oviposition stings were marked (c) larval development inside apple were monitored

Laboratory infested hosts were maintained inside the plastic rearing containers covered by a nylon cloth after oviposition stings were counted in Olympus SZX9 Stereozoom microscope (Fig. 3 (a)). Pupae were collected with a soft forceps, counted and placed in Petri dishes (Fig. 3(b)). Emerged adults were monitored. Additionally, larval feeding behavior and development were evaluated for each host fruits. Experiments were conducted in an

environmental chamber programmed at  $23\pm 2^{\circ}\text{C}$ , 60% RH, with fluorescent lighting providing 16:8 h (light: dark) photoperiod. Several biological parameters were determined as number of oviposition stings per fruit, number of pupa per fruit, number of adults per fruit (male and female) and larval duration. The samples were described with regular averages and Standard errors.

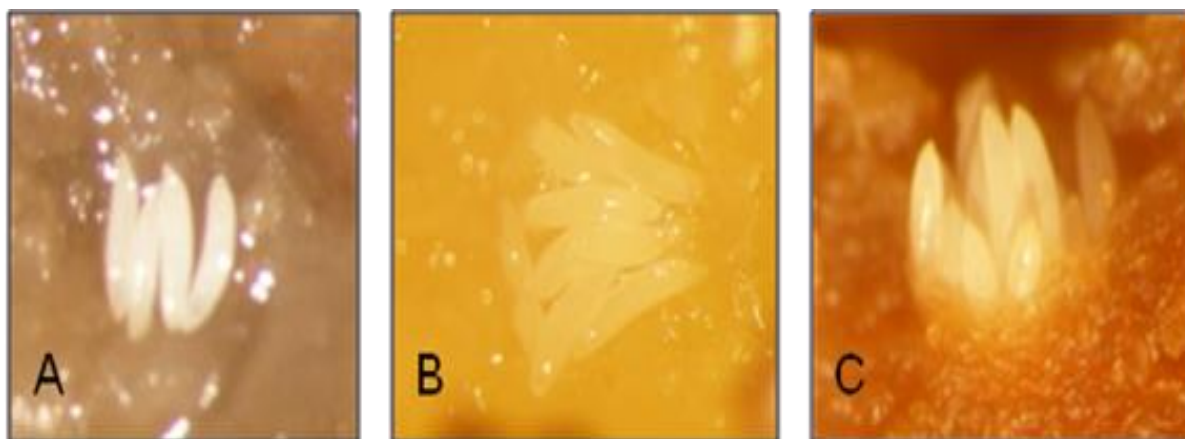


**Figure 3.** A view of tested and cultured Satsuma mandarin (a) infested fruits and (b) monitoring and collecting Medfly pupae.

### Result and Discussion

The infestation and development of the Mediterranean fruit fly larvae and pupae were evaluated on tested hosts in the laboratory. Medfly females laid different number of eggs in each oviposition time. The eggs were deposited inside the hosts as groups of about 4-15 eggs (Fig. 4). They were actually counted as oviposition stings

from the fruit surface. One sting has several eggs but number of oviposition stings were given here as eggs. Medfly female deposited 4 eggs in a pear (Fig. 4(a)), 9 eggs in a peach (Fig. 4(b)) and 10 eggs in a persimmon (Fig. 4(c)). Eggs were in elongated shapes, about  $0.102 \pm 0.006$  mm in width and  $0.476 \pm 0.02$  mm in length. Egg duration was about  $4.02 \pm 1.07$  days in the laboratory conditions.

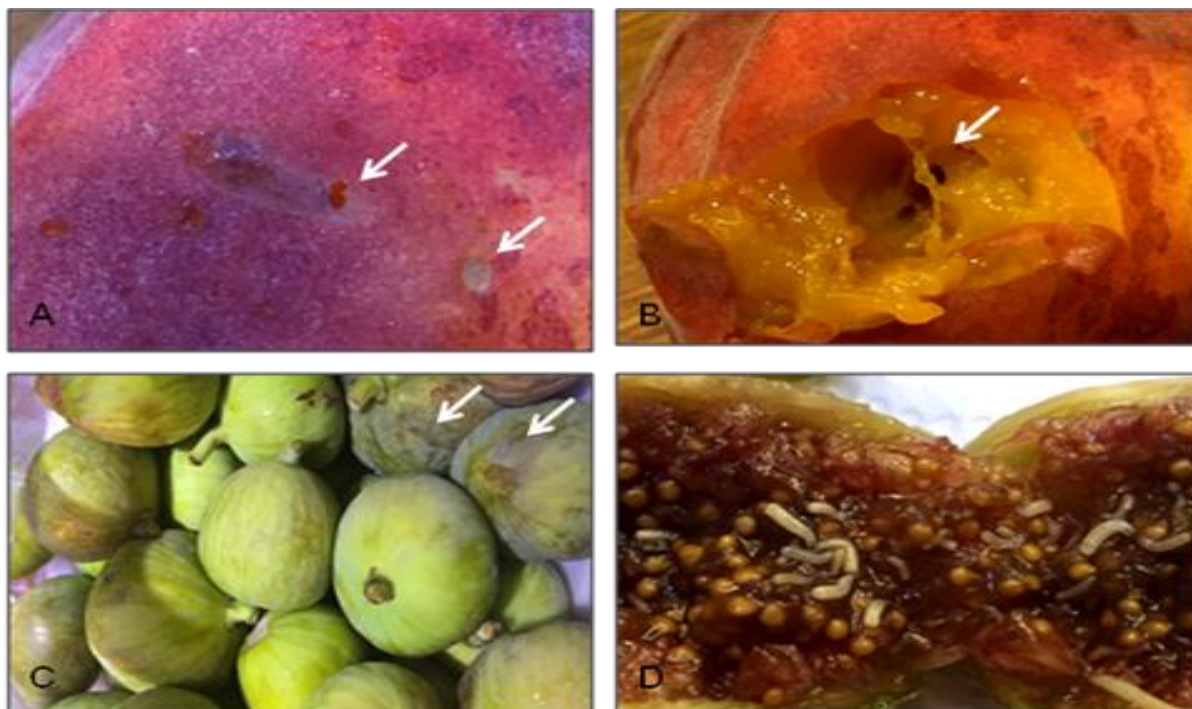


**Figure 4.** Medfly eggs deposited in clusters under the epidermis of different fruits (a) pear, (b) peach and (c) persimmon

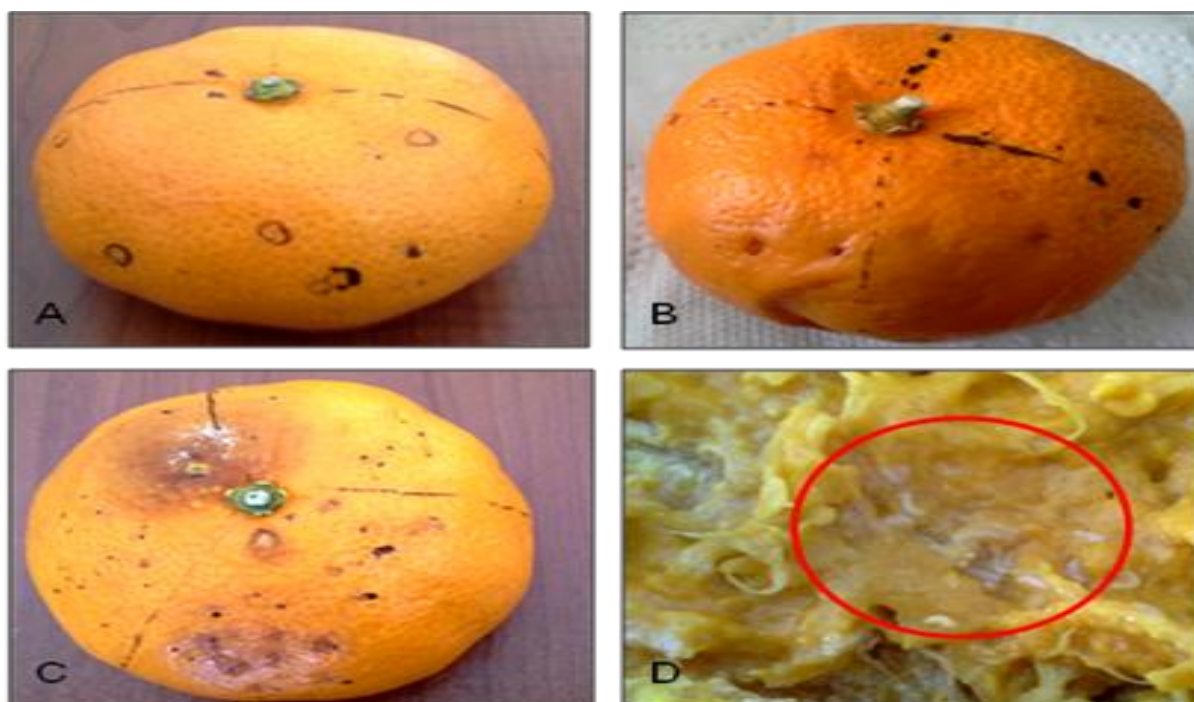
The Medfly has a legless cylindrical shaped larva which expanded at the posterior part. They are creamy-white in color and complete all three instars inside their host. The third instar was about  $5.18 \pm 1.32$  mm in length and the brown mouth parts were obvious. Medfly infestation on peaches (Fig. 5(a) and 5(b)) and figs (Fig. 5(c) and 5(d)) were shown below. Larval feeding became clear if small holes were seen on the host skin of peaches. Larval feeding tunnels were reported when the host was dissected (Fig. 5).

In nature, mandarin and oranges are the primary hosts of the Medfly. The number of

oviposition stings was  $79.33 \pm 13.05$  on Satsuma mandarin and  $58.66 \pm 18.50$  on Murcott mandarin (Table 1). We harvested 81 pupae with 43 adults (29♀, 14♂) from Satsuma mandarin and 39 pupae with 27 adults (8♀, 19♂) from Murcott mandarin. The larval duration was about 21 days on mandarin. Medfly infestation on mandarin was indicated in Fig. 6. Female oviposition stings (Fig. 6 (a)), 7 days after eggs deposited (Fig. 6(b)), 15<sup>th</sup> day of larval development (Fig. 6 (c) and Medfly larval developments inside the mandarin (Fig. 6(d)) were shown.



**Figure 5.** Medfly infestations (a) small holes on peach indicating larval feeding, (b) larval tunnel, (c) infested figs and (d) a dissected fig showed several Medfly larvae



**Figure 6.** Medfly infestations of mandarin (*Citrus reticulata* cv. “Murcott”) (a) marked oviposition stings (b) 7 days after eggs deposited (c) 15<sup>th</sup> day of larval development (d) dissected mandarin showed several Medfly larvae

We tested the pear (*Pyrus pyrifolia*), and two pear cultivars for Medfly infestation. The number of oviposition stings was  $93.00 \pm 38.93$  on pear,  $150.3 \pm 33.02$  on Nashi pear (*Pyrus pyrifolia* Nashi.) and  $120.0 \pm 31.00$  on Deveci pear (*Prunus communis* Deveci) (Table 1). We harvested 2 pupae

with 2 adults (1♀, 1♂) from pear. The larval duration was about 22 days on pear. Even though Medfly deposited many eggs on Nashi pear and Deveci pear, there were no pupae on pear.

Persimmon was the favored host and tested two different times called as Persimmon-1 and

Persimmon-2 for Medfly in the laboratory. The number of oviposition stings was  $88.33 \pm 22.85$  on Persimmon-1 and  $108.0 \pm 19.00$  on Persimmon-2 (Table 1). We harvested 10 pupae with 4 adults (2♀, 2♂) from Persimmon-1 and 23 pupae with 16

adults (8♀, 8♂) from Persimmon-2. There were so many mature larvae feeding inside the Persimmon (Fig. 7) but so fewer pupae were harvested. The larval duration was about 13 days on Persimmon.



**Figure 7.** A dissected persimmon indicated numerous second and third instars feeding inside the fruits

We tested Golden delicious (*Malus domestica* cv. Golden delicious) and Pink lady (*Malus domestica* cv. "Pink Lady") cultivars of apple. The number of oviposition stings was  $24.33 \pm 4.16$ ,  $38.66 \pm 7.23$ ,  $51.50 \pm 3.53$  on Golden delicious 1, 2, 3 and  $21.33 \pm 8.62$  on Pink lady (Table 1). We harvested 51, 16, 73 pupae with 24 adults (12♀, 12♂), 4 adults (2♀, 2♂), 19 adults (11♀, 8♂) from Golden delicious 1, 2 and 3 respectively. The number of pupae was 97 on Pink lady, having 34 adults (21♀, 13♂). Larval duration was  $19.36 \pm 3.95$  on Pink lady (Table 1).

Orange was preferred host for Medfly in nature and tested also in this study. The number of oviposition stings was  $36.00 \pm 6.55$  on oranges, resulted 29 pupae and 11 adults (7♀, 4♂) (Table 1). Larval duration was  $16.20 \pm 2.58$  on orange.

Black plum (*Prunus* sp.) had the highest oviposition sting  $160.3 \pm 33.62$ , having 59 pupae and 22 adults (12♀, 10♂) (Table 1). A tomato (*Solanum lycopersicum* var. *cerasiforme*) was also tested. The number of oviposition stings was  $38.66 \pm 10.21$ , having 10 pupae and 3 females. Larval duration was  $14.00 \pm 3.74$  on plum (Table 1).

**Table 1.** Medfly infestations on different host fruits in the laboratory (Mean±SD)

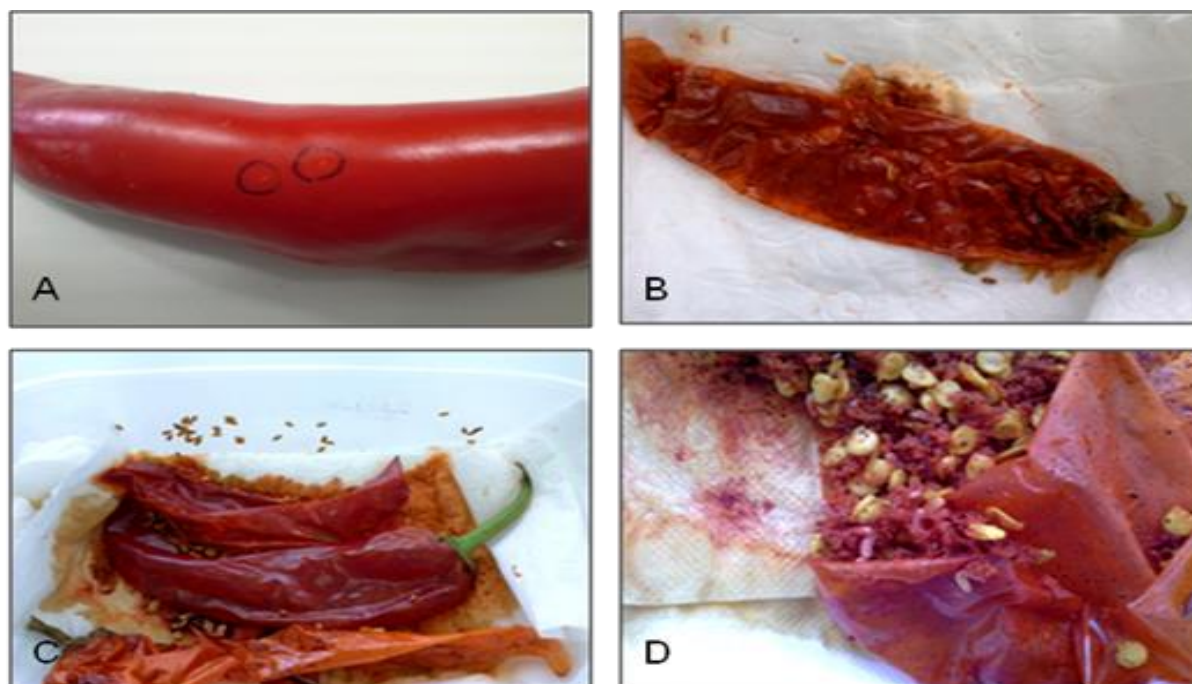
Host	Host Fruit Weight (g)*	No. of oviposition stings **	No. of Pupa	No. of Adults		Larval Duration
				♀	♂	
Satsuma mandarin	69.07±5.06	79.33±13.05	81	29	14	22.11±4.01
Murcott mandarin	60.03±5.55	58.66±18.50	39	8	19	20.33±4.69
Pear	180.0±36.12	93.00±38.93	2	1	1	22.00±2.82
Pear-Nashi	166.2±1.76	150.3±33.02	-	-	-	-
Pear-Deveci	177.9±17.73	120.0±31.00	-	-	-	-
Persimmon -1	200.1±14.97	88.33±22.85	10	2	2	14.80±5.16
Persimmon -2	139.6±14.24	108.0±19.00	23	8	8	11.25±3.61
Apple ( <i>Golden delicious</i> )-1	172.4±10.87	24.33±4.16	51	12	12	26.14±6.72
Apple ( <i>Golden delicious</i> )-2	159.5±14.31	38.66±7.23	16	2	2	17.50±4.81
Apple ( <i>Golden delicious</i> )-3	184.7±0.35	51.50±3.53	73	11	8	25.90±8.16
Apple-Pink Lady	157.0±13.87	21.33±8.62	97	21	13	19.36±3.95
Orange	186.8±17.17	36.00±6.55	29	7	4	16.20±2.58
<i>Mespilus germanica</i> L.	35.67±3.06	4.00±2.64	-	-	-	-
Black Plum	95.05±9.94	160.3±33.62	59	12	10	14.88±4.07
Green pepper1	52.68±6.67	40.00±18.52	74	10	4	15.00±4.39
Pepper-Kapya	73.83±12.33	82.75±16.58	482	65	57	9.50±2.08
Green pepper2	39.26±0.90	13.66±6.42	12	-	1	12.00±2.82
Tomato	38.23±7.51	38.66±10.21	10	3	-	14.00±3.74

\* The mean weight of infested fruits, \*\* The mean number of oviposition stings on each fruit.

We tested green bell pepper (*Capsicum annuum* L) and sweet pepper kapyra cultivar (*Capsicum annuum* L. var. "Kapyra"). The number of oviposition stings was  $40.00 \pm 18.52$  on green pepper-1, having 74 pupae and 14 adults (10♀, 4♂) and  $13.66 \pm 6.42$  stings on green pepper 2, having 12 pupae with 1 male. Pepper cultivar "Kapyra" had  $82.75 \pm 16.58$  stings, having 482 pupae with 122 adults (65♀, 57♂) (Table 1) (Fig. 8). The shortest

larval duration was  $9.50 \pm 2.08$  days on "Kapyra" pepper cultivar.

We tested 10 different fruits and vegetables for laboratory rearing of Medfly and the best host was "Kapyra" pepper. Even though "Kapyra" pepper had an average number of oviposition sting (Table 1 and Fig. 8 (a)), each sting had several eggs so, many Medfly larvae was able to feed on "Kapyra" pepper successfully (Fig. 8(b)) and only the epidermis and seeds were left over from "Kapyra" pepper (Fig. 8(c) and 8(d)).



**Figure 8.** Medfly infestations of red pepper (*Capsicum annuum* L. var. "Kapyra") (a) marked oviposition stings (b) 4 days after eggs deposited (c) 7<sup>th</sup> day of larval development and pupation (d) only epidermis and seeds left from red pepper

Medfly was pupated inside the host or under the rearing containers between the tissue paper (Fig 9(a)). The puparium was about  $3.31 \pm 0.76$  mm long and light to dark brown in color. Pupal duration was about 8-10 days in the laboratory. The adults were yellowish brown in color having obvious yellowish and black spots on thorax (Fig 9 (b)). The legs were light brown and wings were transparent having yellowish brown spots. The abdomen had gray white bands and black hairs. Appearance of Medfly male was like other Tephritids except for a pair of setae close to the eyes (Fig 9(b)). A pheromone produced by males to attract females was shown (Fig 9(c)) and then mating occurred (Fig 9 (d)).

We tested different host fruits for Medfly rearing in the laboratory conditions. The results showed that the oviposition stings were not correlated with fruit weight or pupal yield (Table

1). Black plum had the highest number of oviposition stings ( $160.3 \pm 33.62$ ) with lower pupal yield (59) (Table 1). Nashi pear had the second highest number of oviposition stings ( $150.3 \pm 33.02$ ) but there were no pupal yield (Table 1) of Medfly. So, Nashi pear was checked to see any mature larvae and/or pupae presence. Nashi pear was highly sweet and extremely juicy; there were about 57 drowned or dead larvae inside which were not able to exit to pupate. Although, the slices of Nashi pear were used for adult food in the cages (Fig. 1), it was not an appropriate for larval host.

Oviposition stings were indicated that Medfly females deposited reasonable amount of eggs inside the "Kapyra" pepper which had the highest number of larval survival and pupal yield (Table 1) and the best host for laboratory rearing.

In nature, female decide a host to deposit eggs for further generations which is associated with coevolution between host and polyphagous insects (Janz, 2002; Malacrida et al. 1998; Thompson and Pelmyr, 1991; Genc, 2016). According to Edwards and Wratten, 2016, polyphagous insects select their plant hosts based on nutritional values. In the present study, Medfly showed different ovipositional and larval feeding preferences. Host preferences were associated with larval feeding and completing their immature

stages. It was not related with number of deposited eggs by females (Table 1). In other words, fruits having more eggs were not the preferred host. Some previous studies also referred that there were no correction between oviposition stings and larval performance (Silva and Zucoloto, 1993; Janz, 2002). Besides, some studies reported that Medfly had no ovipositional preferences for the nutritional parts in the tested host (Kolbe and Eskafi, 1989).



**Figure 9.** Medfly pupae and adult stage (a) pupae (b) mating adults (c) adults secrete a pheromone before mating (d) oviposition of Medfly adults in persimmon

This work indicated that the number of eggs or oviposition stings was not related with larval survival or harvested pupae. Even though some tested hosts were chosen by females and deposited several eggs, larval feeding and survival were high at the beginning but decreased when they became mature larvae or pupae. Laboratory testing of several different fruits and vegetables suggested that “Kapyra” pepper had the higher larval survival with moderate oviposition stings. These results were also correlated with some previous studies (Thompson and Pelmyr, 1991; Kolbe and Eskafi, 1989). Therefore, female’s oviposition behavior and the larval development are very important for fruit fly infestation.

### Conclusion

In the present study, host preferences and infestations of Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) were determined by testing different fruit and vegetable

cultivars in the laboratory conditions. The results showed that Medfly females prefer to choose sweeter hosts such as Black plum or Nashi Pear for oviposition and “Kapyra” pepper was the most acceptable host for larval survival and development.

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