

## IMPACTS OF THE VARIETY OF POMEGRANATE FRUIT ON PREFERENCE AND PERFORMANCE OF *ECTOMYELOIS CERATONIAE* (PYRALIDAE) AND *DEUDORIX LIVIA* (LYCAENIDAE)

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(Received 23<sup>rd</sup> Jul 2024; accepted 7<sup>th</sup> Jan 2025)

**Abstract.** In Tunisia, pomegranate (*Punica granatum* L.) is an important crop both economically and culturally. The marketing quality of pomegranate fruit is mainly affected by two pests namely *Ectomyelois ceratoniae* (Zeller) and *Deudorix livia* (Klug). The present work aims to assess the biological parameters and life history variables of immature and adult stages of both pests together with their larval competition under controlled laboratory conditions ( $T = 30 \pm 1^{\circ}\text{C}$ ;  $HR = 70 \pm 5\%$ ; 16L: 8D photoperiod). The rearing was carried out on three pomegranate varieties namely Guebsi, Tounsi and Gares collected from Gafsa oasis (Southwestern, Tunisia). Results indicated that the shortest larval development time of *E. ceratoniae* (31.9 days) was observed in Tounsi variety. Besides, the shortest larval period of *D. livia* was registered in Guebsi variety (23 days). Moreover, the lowest survival rate (around 40%) was observed in the first larval stage with all the pomegranate varieties used for insect rearing. For *D. livia*, the lowest survival rate was observed in the first (48%) and the second (52%) instars feeding on the Gares variety and in the fourth instar rearing on the Guebsi variety (55%). Under competitive conditions the larvae weights were lower than those obtained when larvae were reared separately.

**Keywords:** *Ectomyelois ceratoniae*, *Deudorix livia*, oasis, biological parameter, rearing

### Introduction

Pomegranate, *Punica granatum* L. (*Punicaceae*), is an important commercial fruit crop that is well cultivated in parts of Asia, North Africa, the Mediterranean and the Middle East (Sarkhosh et al., 2006). The tree and its uses are found in many ancient cultures as a food and as a medicinal remedy (Sarkhosh et al., 2020). Pomegranate is recognized as a remedy to soothe stomach ailments (Ajaikumar et al., 2005). It is also used as a natural source of phenolic compounds, sugars, proteins and minerals (Elfalleh et al., 2009). The edible flesh of the pomegranate is often eaten fresh or processed into juice. The juice can be processed into beverages, wine, syrup and sauces in some communities (Elfalleh et al., 2009). It has been reported that extracts from different parts of the pomegranate fruit such as peel, juice and seeds have high

antioxidant capacity (Elfalleh et al., 2009; Gil et al., 2000; Aviram et al., 2000; Singh et al., 2002).

The carob moth, *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae), has been identified as one of the most destructive pests of pomegranates and other fruits such as dates, almonds, carob, walnuts, figs and pistachios, with a wide distribution in many tropical and subtropical regions (Warner et al., 1990; Mediouni, 2005; Ranjbar et al., 2011; Kishani-Farahani et al., 2012; Soufbaf et al., 2018). In Tunisia, pomegranates are the main hosts of the carob moth *E. ceratoniae*. In this context, Mediouni and Dhoubi (2007) reported that the annual infestation rate of pomegranates can be up to 80%. The larvae of *E. ceratoniae* feed inside the fruit, leading to contamination with saprophytic fungi and causing extensive damage during the growing season and after harvest, rendering the fruit unfit for human consumption (Shakeri, 2004). In addition, the pomegranate fruit butterfly *Deudorix* (*Virachola*) *livia* was first detected in Tunisia in 2006, causing severe damage (52%) to pomegranates in the south of the country (Gabes region) (Ksentini et al., 2011). Other studies conducted in Tunisia showed that *D. livia* causes noticeable production losses in pomegranates (Ksentini et al., 2010; Braham, 2015). In addition, *D. livia* has been reported as a serious pest of pomegranate worldwide, for example in Oman (Abbes et al., 2008; Kinawy et al., 2008), Jordan (Obeidat and Akkawi, 2002), Egypt (Sayed et al., 2010; El-Solimany and Negam, 2023), Saudi Arabia (El-Hawagry et al., 2016), Algeria (Beladis et al., 2018) and Iran (Alotaibi et al., 2022).

Recent research pointed out that there is different preference levels among some studied pomegranate varieties (Hamad et al., 2024). Among the most sustainable methods for controlling insect pests, the use of resistant varieties appears to be sustainable (Golizadeh et al., 2009; Soufbaf et al., 2010; Golizadeh and Abedi, 2016). The correct use of resistant varieties in plant protection requires knowledge of the life cycle parameters and biological variables of the pests (Nawrot et al., 2010; Golizadeh and Abedi, 2017; Karimi-Pormehr et al., 2018; Gvozdenac et al., 2018). However, little is known about the life cycle and reproductive parameters of *E. ceratoniae* and *D. livia* on pomegranates in Tunisia except studies conducted by Ksentini et al. (2011) and Gharbi (2010).

To develop effective management against *E. ceratoniae* and *D. livia*, the current study was planned to evaluate impacts of the variety (Tounsi, Guebsi and Gares) of pomegranate fruit on the preference and performance of these pests and to study their life cycle, biological parameters and larval competitiveness.

## Materials and methods

### *Pomegranate varieties*

The fruits of three varieties of pomegranate (*Punica granatum* L.), including Guebsi, Tounsi and Gares, were collected when ripe from trees in the Gafsa oasis in southwestern Tunisia (34°23'39"N8°45'50"E). The ripe, fresh and healthy fruits were selected and transported in plastic pots to the laboratory where they were stored at 4°C in the refrigerator to be used as food for the larvae.

### *Insect rearing*

Rearing was performed in rectangular boxes (10 × 9.5 × 6 cm) under the following controlled conditions (T = 30 ± 1°C, HR = 70 ± 10%, 16L: 8D photoperiod) according to the protocol described by Zare et al. (2013).

To begin rearing, different larval stages of *D. livia* and *E. ceratoniae* were collected from infested fruits of the three pomegranate varieties. The larvae were separated according to their developmental stages. The larvae were fed with pomegranate fragments. The pupae were placed in cylindrical cages containing the pomegranate fruit. The cages were kept under the same rearing conditions until the adults developed. The newly hatched adults were offered a sugar solution soaked in pieces of cotton. After mating (24 h) and egg hatching, thirty fertile eggs were used for observation in the laboratory to study the life history and reproductive parameters of *E. ceratoniae* and *D. livia*. The eggs were counted until the female stopped laying eggs. After the larva died, it was replaced by a new first larva to reach the further stages until pupation. The observations continued until the insects died. The distinction between fertilized and unfertilized eggs is based on their color, as described by Gharbi (2010). The color of the fertilized egg in *D. livia* and *E. ceratoniae* is green-gray and pink, respectively, while the color of the unfertilized egg is always white.

### ***Life history variables***

Based on the individual rearing of the different stages of the two insects, the following parameters were investigated:

- Egg incubation Period (IP): time between egg deposition and hatching of the eggs
- Larval development period (LP): duration of the successive larval stages
- Total larval period (TLP)
- Pre-pupal and pupal periods (PPP, PP)
- Sex ratio: the percentage of females in the population
- Longevity: duration of survival of males and females
- Survival rate (%), recorded per stage

### ***Reproductive biology of E. ceratoniae and D. livia***

In this study, the pre-oviposition period (POP: the time between the emergence of an adult female and the start of her oviposition), the oviposition period (OP) and fecundity (eggs laid during the reproductive period) were recorded. The oviposition boxes were observed every 24 h, and the observations were continued until the death of the insects.

### ***Laboratory larval competition between the two insects***

This study was conducted under controlled laboratory conditions ( $T = 30 \pm 1^{\circ}\text{C}$ ,  $\text{HR} = 70 \pm 10\%$ , 16L: 8D). The aim of the study was to investigate the competition between the third instar larvae of the two insects until pupation. A larval ratio of 50% *E. ceratoniae* to 50% *D. livia* was used for this study. The choice of this ratio was based on personal observations in the fields. The experiments were conducted in five replicates. The larvae of both insects were housed in glass Petri dishes (100 × 20 mm) and fed with 6 pomegranate seeds (2 g) of the Guebsi variety, which were placed in the incubator under the same rearing conditions. Since this variety was less suitable for both *E. ceratoniae* and *D. livia* during laboratory rearing, this cultivar was chosen as the food source to create a forced competition for survival. Competition was monitored by measuring the weight of the larvae and pupae. The pomegranate seeds were replaced every 48 h.

## Statistical analysis

Statistical analyses were performed using the SPSS statistical program version “20”. The biological parameters of *E. ceratoniae* and *D. livia* on three pomegranate varieties were compared at  $P \leq 0.05$  using one-way ANOVA followed by Tukey-test.

## Results

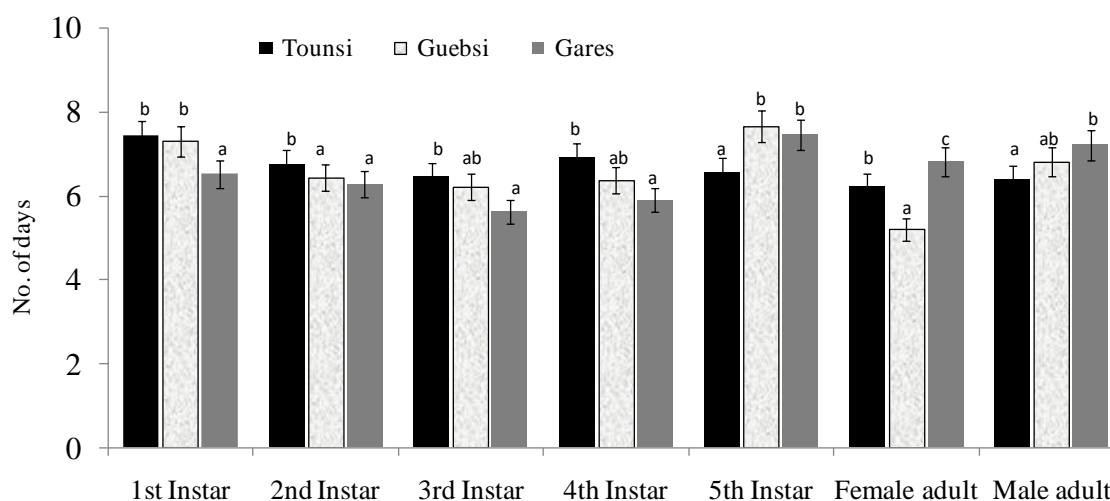
### Life history and reproductive biology of *E. ceratoniae*

#### Incubation period

The average duration of incubation of the eggs in the three varieties studied is shown in Figure 2. The female lays oval, whitish eggs. As soon as the egg is fertilized, its color changes to pink. There were significant differences in the mean incubation period between the pomegranate varieties. Our results (Fig. 2) show that the longest egg incubation period was observed in the Guebsi and Tounsi varieties (7.58 and 7.43 days on average, respectively) and the shortest in the Gares variety (6.81 days on average).

#### Larval periods

Five larval stages (instars) were observed in *E. ceratoniae*. The duration of the individual stages is described below (Fig. 1):



**Figure 1.** Life duration of each stage of *E. ceratoniae* under laboratory condition (30°C, 70 ± 10% Relative humidity). Bars for the duration of each stage of *E. ceratoniae* in different varieties that are labeled with different lower-case letters (a, b) are statistically different according to Tukey’s test ( $P \leq 0.05$ )

#### First instar

The first stage larvae (L1) of *E. ceratoniae* were pink and had a brownish anal part. Our results showed that the average duration of the first larval stage was 7.42, 7.3 and 6.52 days for the Tounsi, Guebsi and Gares varieties, respectively.

## Second instar

In the second instar, the larva spent 6.75, 6.43 and 6.26 days on the varieties Tounsi, Guebsi and Gares, respectively.

## Third instar

The duration of the third stage was 6.45, 6.21 and 5.62 days on Tounsi, Guebsi and Gares varieties respectively.

## Fourth instar

During the fourth larval instar, the sexual dimorphism between males and females of *E. ceratoniae* is clear. The male caterpillar is characterized by a brownish spot on the lower back, which corresponds to the male gonads. The duration of this stage was 6.9, 6.36 and 5.9 days for the Tounsi, Guebsi and Gares varieties, respectively.

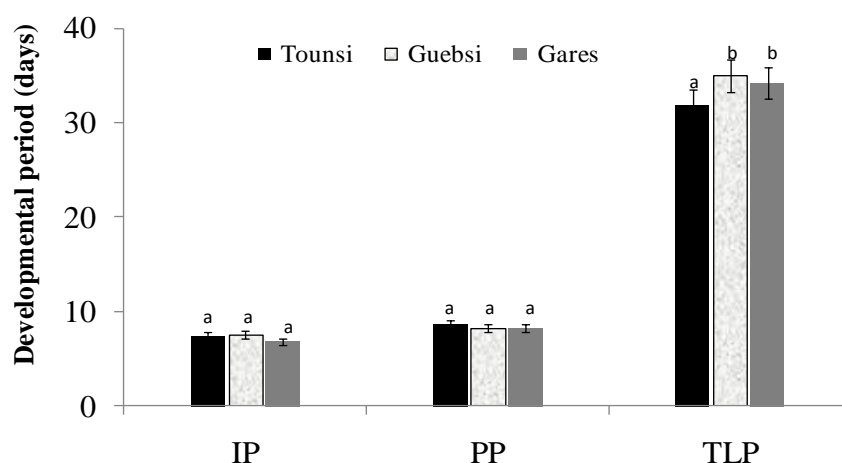
## Fifth instar

At this stage, the larvae are yellowish in color. The average duration of the fifth larval stage was 6.56, 7.66 and 7.45 days for the varieties Tounsi, Guebsi and Gares, respectively.

Our results (Fig. 2) showed that the total duration of larval stages varies depending on the variety used for feeding. The longest larval period was observed in the Guebsi variety with a mean value of 35 days, while the shortest period was observed in the Tounsi variety with a value of 31.9 days.

## Pupa stage

At this stage the pupae are brown. Male and female pupae differ in the position of the virtual genital pore, which is much further forward in females than in males. The average duration of this stage (Fig. 2) is comparable in all three varieties. It is about 8.61, 8.28 and 8.25 days for the Tounsi, Guebsi and Gares varieties respectively.



**Figure 2.** Egg incubation period (IP), total larval period (TLP) and pupation period (PP) (days) of *E. ceratoniae* among the three pomegranate varieties. Developmental periods were compared using one-way ANOVA ( $P \leq 0.05$ ). Bars of each developmental stage of *E. ceratoniae* in different varieties that are labeled with different lower-case letters (a, b) are statistically different according to Tukey's test ( $P \leq 0.05$ )

## Adults

Adult *E. ceratoniae* is light gray. The sexual dimorphism can be seen at the end of the abdomen, where the females have a small, relatively dark circular depression from which the ovipositor emerges. The males have two externally visible valves. The females are also larger than the males and have a more voluminous abdomen. The longevity of both male and female adults of *E. ceratoniae* was lowest when larvae were reared on the Guebsi strain (Fig. 1). The longevity of adult males ranged from 6.4 to 7.2 days, while the longevity of females ranged from 5.2 to 6.8 days.

## Sex ratio

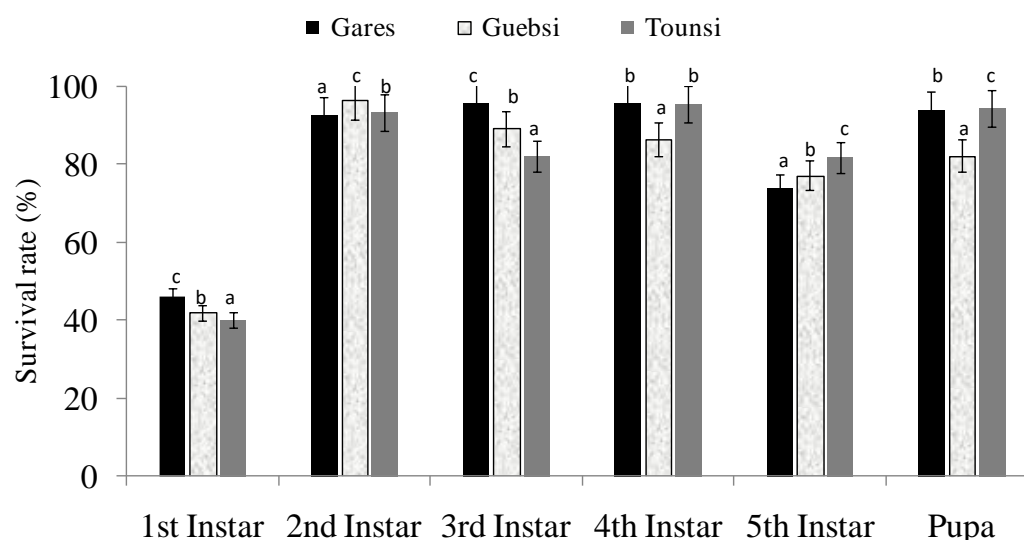
The adults obtained from the rearing of *E. ceratoniae* allowed us to collect a total of 34 males and 31 females, which corresponds to a sex ratio of 1. Our results (Table 1) show that *E. ceratoniae* has the highest sex ratio in the Guebsi variety (1:1.2), while the lowest was observed in the Gares variety (1:0.91).

**Table 1.** Sex ratio of *E. ceratoniae* fed on the three pomegranate varieties

Varieties	Tounsi	Guebsi	Gares
No. Male adults	11	12	11
No. Female adults	11	10	10
Sex-ratio	1:1	1:1.2	1:1.1

## Survival rate (%) / stage

The survival rates (%) for each larval stage of *E. ceratoniae* and for the pupal stage are shown in Figure 3. The lowest survival rate was observed in the first larval stage for all pomegranate varieties used for insect rearing.



**Figure 3.** Survival rate (%) / development stage of *E. ceratoniae* rearing on the three pomegranate varieties (Tounsi, Guebsi and Gares). Survival rate of each stage was compared using one-way ANOVA ( $P \leq 0.05$ ). Bars for survival rate (%) of each stage of *E. ceratoniae* in different varieties that are labeled with different lower-case letters (a, b, c) are statistically different according to Tukey's test ( $P \leq 0.05$ )

### ***Reproductive biology of E. ceratoniae on three pomegranate varieties***

The data on pre-oviposition period (POP), oviposition period (OP) and fecundity of adult *E. ceratoniae* on the tested pomegranate varieties are shown in Table 2.

**Table 2.** Biological parameters of *E. ceratoniae* adults reared on three pomegranate varieties (Tounsi, Guebsi, Gares)

Varieties	Tounsi	Guebsi	Gares
POP (days)	1.6 ± 0.89a	1 ± 0.00a	1.2 ± 0.44a
OP (days)	3.4 ± 1.14a	3 ± 1.22a	4.4 ± 0.54a
Fecundity	75.66 ± 2.93c	73.5 ± 1.73b	33.5 ± 2.50a

Data are means ± SD. For each biological parameter, different lower-case letters within each column indicate significant differences between varieties according to Tukey's test at  $p \leq 0.05$

#### ***Pre-oviposition period (POP)***

We found that pre-oviposition period (POP) varied considerably depending on the pomegranate varieties used as food source. The longest POP was observed with the Tounsi variety (1.6 days) and the shortest with the Guebsi variety (1 day).

#### ***Oviposition period (OP)***

The oviposition period was longest when the females of *E. ceratoniae* were reared on the Gares variety and shortest when they were reared on the Guebsi variety.

#### ***Fecundity***

Mean number of eggs laid by females during the reproductive period. The fecundity of *E. ceratoniae* varied as a function of the three pomegranate varieties tested; the highest value was observed on Tounsi variety (75.66 eggs) and the lowest was on Gares (33.5 eggs).

### ***Life history and reproductive biology of D. livia***

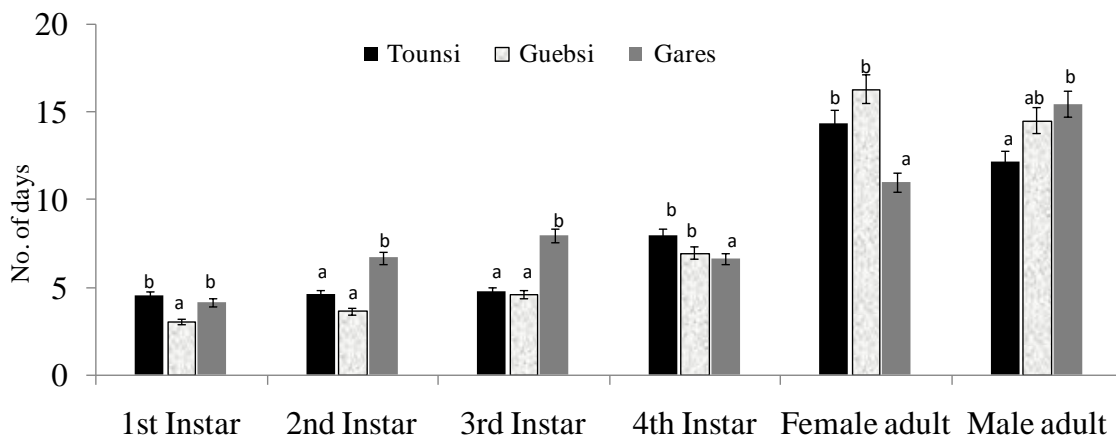
#### ***Incubation period***

Observation of sampled pomegranate fruits showed that *D. livia* eggs are deposited preferentially on mature and winter fruits. The calyx is the preferred site for egg deposition on winter fruit. On mature fruit, eggs are deposited near the calyx, in the middle of the fruit, at the bottom and very rarely on the peduncles or leaves. Our results (Fig. 5) showed that the longest egg incubation period was showed on the Gares and Tounsi varieties (an average of 6.43 and 6.31 days respectively), while the shortest was observed on Guebsi variety (an average of 5.81 days).

#### ***Larval periods***

The larvae hatched from the eggs are used for rearing *D. livia* larvae on the three varieties under controlled conditions. This rearing allowed us to follow the development of the different stages and determine their duration. The observations showed that the individuals complete their larval development in 4 stages. The observation of the development of the larval stages showed that the duration of the individual stages varies

depending on the type of feeding. The average duration of the individual stages is shown in *Figure 4*.



**Figure 4.** Life duration of each stage of *D. livia* under laboratory condition (30°C, 70 ± 10% Relative humidity). The duration of each stage was compared using one-way ANOVA ( $P \leq 0.05$ ). Bars for the duration of each stage of *D. livia* in different varieties that are labeled with different lower-case letters (a, b) are statistically different according to Tukey's test ( $P \leq 0.05$ )

#### First instar

After hatching from the egg, the caterpillar is black; the anus is gray and quickly turns brownish when exposed to light. The average duration of the first larval instar was 4.52, 3.09 and 4.16 days for the Tounsi, Guebsi and Gares varieties, respectively.

#### Second instar

The duration of the second larval stage in the Tounsi, Guebsi and Gares varieties was 4.64, 3.68 and 6.71 days respectively.

#### Third instar

The larvae turned pink (the color of pomegranate seeds). The duration of the third stage was 4.76, 4.62 and 8 days for the Tounsi, Guebsi and Gares varieties respectively.

#### Fourth instar

The duration of the last larval stage was 8, 7 and 6.66 days for the varieties Tounsi, Guebsi and Gares, varieties respectively. Our results showed that the total duration of the larval stages of *D. livia* varied depending on the cultivar. For the Tounsi, Guebsi and Gares cultivars, the duration was 24, 23 and 30 days, respectively.

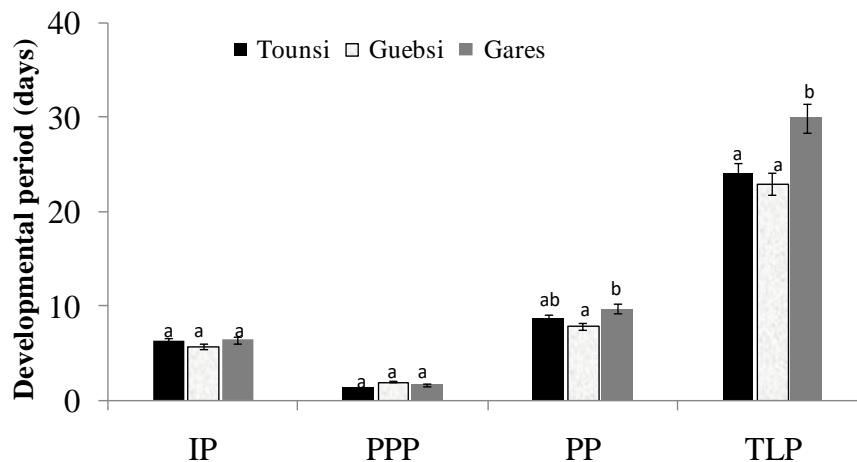
#### Pre-pupation stage

At the end of its development, the larva is firmly attached and has stopped feeding. This is the prepupal stage, which is short and characterized by the whitish color of the prepupa. The duration of this stage was between 1 and 2 days, with average values of 1.38, 2 and 1.67 days for the Tounsi, Guebsi and Gares varieties (*Fig. 5*).



### Pupation stage

The pupa has a light color and then turns brown. The duration of this stage varies between 7 and 12 days, with average values of 8.64, 7.91 and 9.75 for the Tounsi, Guebsi and Gares varieties respectively (Fig. 5). At this stage, a distinction can be made between male and female pupae. In fact, the male pupae are smaller than the female pupae.



**Figure 5.** Egg incubation period (IP), total larval period (TLP), pre-pupation period (PPP) and pupation period (PP) durations (days) of *D. livia*, on Tounsi, Guebsi and Gares pomegranate varieties. Developmental periods were compared using one-way ANOVA ( $P \leq 0.05$ ). Bars of each developmental stage of *D. livia* in different varieties that are labeled with different lower-case letters (a, b) are statistically different according to Tukey's test ( $P \leq 0.05$ )

### Adult

At this stage, the adult emerged from the pupa and the two sexes are very different from each other. The male is medium-sized and has an orange-golden color. The corners of the forewings are dark brown, with a tail near a black spot on each wing. The underside of the wings is grayish with white color on black or brown. There are small black spots at the base of each hind wing. The females are golden in color with blue spots on the wings. Each wing has black spots that are fused with the tail. This looks like an insect head with eyes and antennae, the characteristic morphological features of the insect.

The longevity of adult females of *D. livia* was lowest (11 days) when the larvae were reared with the Gares strain, and the lowest longevity of adult males (12.2 days) was achieved when the larvae were reared with the Tounsi strain (Fig. 4).

### Sex ratio

When monitoring the emergence of *D. livia*, we were able to collect a total of 33 males and 33 females, which corresponds to a sex ratio of 1 (Table 3). The highest sex ratio was observed in the Tounsi variety, while the lowest was recorded in the Guebsi variety.

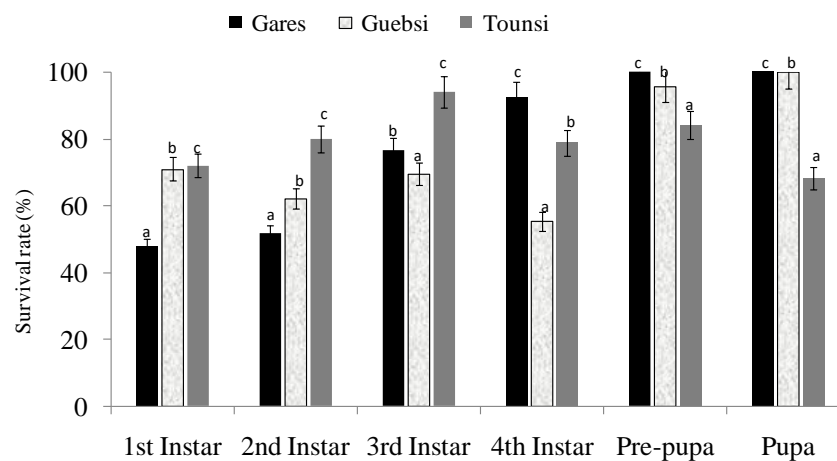
### Survival rate (%)/stage

The survival rates (%) for each stage of *D. livia* are shown in Figure 6. The lowest survival rate of *D. livia* was observed in the first and second larval stages when fed with the Gares variety (48 and 52%) and in the fourth larval stage when reared with the

Guebsi variety (55%). However, the highest survival rate was observed in the pupae that emerged from larvae that fed on both varieties.

**Table 3.** Sex ratio of *D. livia* fed on Tounsi, Guebsi and Gares pomegranate varieties

Varieties	Tounsi	Guebsi	Gares
No. Male adults	17	10	6
No. Female adults	16	11	6
Sex-ratio	1:1.06	1:0.90	1:1



**Figure 6.** Survival rate (%)/development stage of *D. livia* reared on Tounsi, Guebsi and Gares pomegranate varieties. Survival rate of each stage was compared using one-way ANOVA ( $P \leq 0.05$ ). Bars for survival rate (%) of each stage of *D. livia* in different varieties that are labeled with different lower-case letters (a, b, c) are statistically different according to Tukey's test ( $P \leq 0.05$ )

### Reproductive biology of *D. livia* on three pomegranate varieties

The observations on the pre-oviposition and oviposition time and on the fecundity of the adult *D. livia* on the three pomegranate varieties are listed in Table 4.

#### Pre-oviposition period (POP)

The results showed that the duration of the pre-oviposition period (POP) varied depending on the pomegranate varieties used as food source. The POP was longest for the Tounsi variety (6.14 days) and shortest for the Gares variety (4.10 days).

#### Oviposition period (OP)

This period was longest when the females of *D. livia* were reared on the Guebsi variety (7.22 days) and shortest when they were reared on the Gares strain (3.33 days).

#### Fecundity

The fecundity of *D. livia* females varied depending on the three pomegranate varieties; the highest fecundity was observed in the Tounsi variety ( $19.42 \pm 2.29$  eggs) and the lowest in the Gares variety ( $10.75 \pm 1.70$  eggs).

**Table 4.** Biological parameters of *D. livia* adults reared on Tounsi, Guebsi and Gares pomegranate varieties

Varieties	Tounsi	Guebsi	Gares
POP (days)	6.14 ± 1.86a	5.09 ± 0.53a	4.10 ± 1.51a
OP (days)	6.22 ± 1.56b	7.22 ± 2.53ab	3.33 ± 0.51a
Fecundity	19.42 ± 2.29c	16.66 ± 1.15b	10.75 ± 1.70a

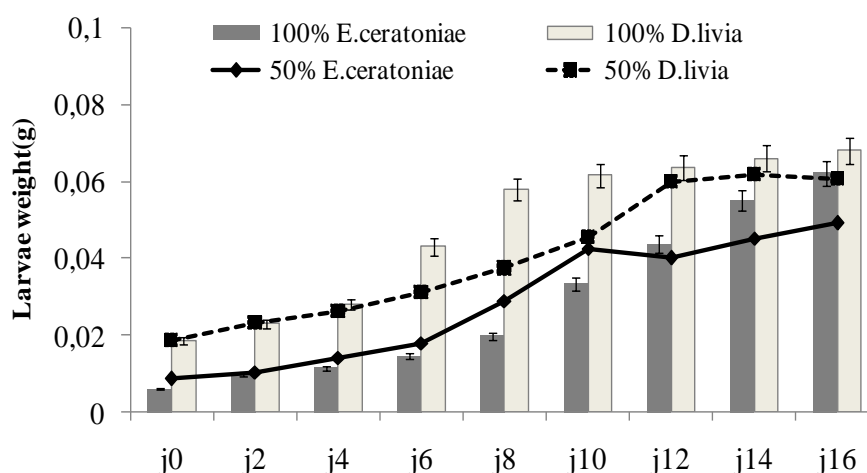
Data are means ± SD. For each biological parameter, different lower-case letters within each column indicate significant differences between varieties according to Tukey's test at  $p \leq 0.05$

## Larval competition

### Effect of competition on larvae weight

When comparing the weight development of the larvae under conditions of competition with equal density (50/50%), we found that the weight of both *E. ceratoniae* and *D. livia* increased when a competing larva was present.

When observing this competition, we started with the third-stage larvae until the last stage, and we found that the highest weight was observed in the fifth and fourth larval stages of *E. ceratoniae* (0.049 g) and *D. livia* (0.06 g), respectively. The results in Figure 7 show that the larval weight of both pests increased when they were not competing. We found that the larval weight of *D. livia* was higher than that of *E. ceratoniae*.



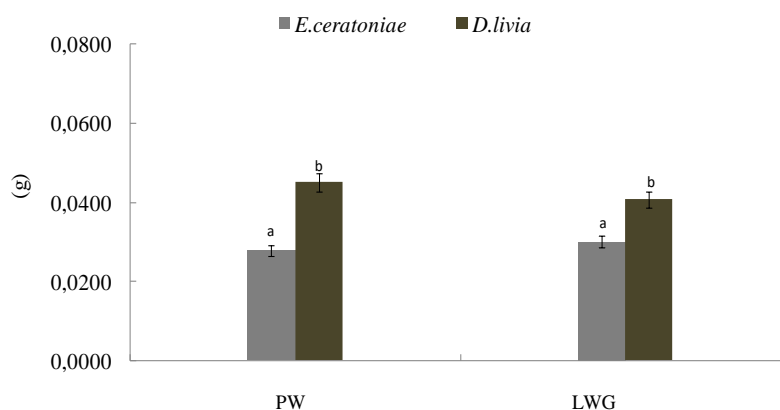
**Figure 7.** Evolution of larvae weight without competition (100% *E. ceratoniae* and 100% *D. livia*) and under a competition of 50%-50% of *E. ceratoniae* -*D. livia*. The mean larvae weight of the studied pests were compared using one-way ANOVA ( $P \leq 0.05$ )

### Effect of competition on pupae and larvae weights of *E. ceratoniae* and *D. livia*

The data on pupal weight (PW) and larval weight gain (LWG) with 50% *E. ceratoniae*–50% *D. livia* competition on the tested pomegranate cultivar are shown in Figure 8.

Our results showed that the pupae of *D. livia* larvae had the highest weight (0.045 g) compared to those of *E. ceratoniae* larvae (0.027 g). For larval weight, the values

increased with a difference corresponding to weight gain of 0.030 g for *E. ceratoniae* and 0.040 g for *D. livia*.



**Figure 8.** Pupae weight (PW) and larvae weight gain (LWG) under a competition of 50% *E. ceratoniae*-50% *D. livia*. The pupae weight and larvae weight gain of the studied pests were compared using one-way ANOVA ( $P \leq 0.05$ ). Bars for the pupae weight and larvae weight gain that are labeled with different lower-case letters (a, b) are statistically different according to Tukey's test ( $P \leq 0.05$ )

## Discussion

Our results show that the duration of the developmental stages of both *E. ceratoniae* and *D. livia* varies depending on the host species and larval stage.

For *E. ceratoniae*, the first and last larval stages were the longest with an average of 7 days, while the third larval stage was the shortest with only 5-6 days. The results of the present study show that *E. ceratoniae* can lay up to 73.5 eggs and pass through five larval stages under laboratory rearing conditions. A previous study found that *E. ceratoniae* can lay between 36.91 and 82.74 eggs and pass through five larval stages (Abedi et al., 2019). In addition, Kumar (2014) found that the fecundity of *E. ceratoniae* is 27.4 eggs. We found that the total duration of larval stages ranged from 31.9 to 35 days in three species. The effect of host species on larval duration has been studied by many researchers. The larval development time of *E. ceratoniae* varied greatly from 24.88 to 72.9 days depending on the host plant (Norouzi et al., 2008).

The larval period determined by Kumar (2014) was 37.8 days on average. Pupation time was 8.61, 8.28 and 8.25 days for Tounsí, Guebsi and Gares varieties, respectively. Our results showed higher values than those reported by Norouzi et al. (2008) (7.08 days for pomegranate) and Mehrnejad (1992) (7.18 days for pistachio), but lower than those reported by Kumar (2014) (9.8 days). In this study, the range of male and female longevity of *E. ceratoniae* was higher than three pomegranate cultivars including Malase-danesyah, Gabri and Shahvar (Zare et al., 2013) and eleven commercial pomegranate cultivars (Abedi et al., 2019).

The results of the present study show that *D. livia* passes through four larval stages. This finding agrees with the results of Awadallah et al. (1970), Gharbi (2010), and Almi et al. (2018). Other results were noted by Mkaouar and Ben Djamaa (2016) who reported five larval stages. This difference is probably due to the different growth conditions as well as the feeding host plants (Almi et al., 2018). Among the larval

stages, the fourth stage of *D. livia* was the longest with an average of 8 days, while the prepupal stage was the shortest. Our results showed that *D. livia* can lay up to 19.42 eggs. In this context, Gharbi (2010) found that the female in laboratory rearing can lay up to 13.1 eggs/fruit, which is less than our results. The incubation period lasts between 4-7 days. In addition, Almi et al. (2018) showed that the incubation period varies between 5 and 9 days. Similarly, Gharbi (2010) recorded duration of 5 days. However, this duration lasted between 6 and 25 days at 25°C and 4 days at 30°C (Awadallah et al., 1970). Studies on other hosts showed that the incubation period of *D. livia* on guava lasts 8-10 days, with a mean duration of 8.8 days (Khan, 2016). The total duration of the larval stages of *D. livia* ranged from 22.55 to 24 days, which is very close to the results of Gharbi (2010) with a mean duration of 25.94 days. This result differs from that of Almi et al. (2018), which is between 17 and 10 days under ambient conditions. The prepupal stage lasts an average of two days and the insect gradually transforms into a pupa. The average pupal duration ranged between 7 and 12 days, with an average of 8.64, 7.91 and 9.75 for the Tounsi, Guebsi and Gares varieties, respectively. Similarly, Almi et al. (2018) found that the pupal stage lasts 6 to 9 days, with an average of 7.18 days. The duration of each stage is probably related to climatic conditions, photoperiodism and the type of food; larval development depends on their ability to quickly accumulate reserves to initiate the pupal stage (Almi et al., 2018). The value of the sex ratio, which is of the order of 1, indicates a balanced population in which the females are slightly more numerous than the males.

The highest mortality rate of *E. ceratoniae* was observed in the first larval stage and amounted to 60% in all pomegranate varieties used for insect rearing. *Deudorix livia* showed the highest rate (more than 50%) in the first and second larval stages when feeding on the Gares variety. For the larval competition, no data on the weight of larvae and pupae of *E. ceratoniae* and *D. livia* during the laboratory competition are available to allow a comparison. However, Abedi et al. (2019) found that the weight of the fifth larval instar and pupae of *E. ceratoniae* varied considerably depending on the pomegranate variety. The mean weight of fifth instar larvae varied between 0.042 g and 0.0615 g, which is close to our results. In addition, the pupal weight values varied between 0.034 g and 0.044 g (Table 2), which is significantly higher than our results.

The results showed that Tounsi was a relatively susceptible and more suitable pomegranate cultivar than Guebsi and Gares, which are the least suitable (most resistant) cultivars for *E. ceratoniae* infestation. However, Tounsi and Guebsi were relatively susceptible to *D. livia* compared to Gares, which was the most resistant. These differences in the susceptibility or resistance of fruits are apparently due to the higher content of secondary metabolites (Abedi et al., 2019; Soliman, 2020) and the chemical composition of pomegranate fruits (Hamad et al., 2024). This finding could be useful in the development of integrated pest management strategies for these pests.

## Conclusions

The results of the present work showed the preference of *E. ceratoniae* and *D. livia* for the consumption of different pomegranate varieties. Our results indicated that the larvae of *E. ceratoniae* and *D. livia* that fed on the Guebsi and Gares varieties displayed the longest larval period. Therefore, the Guebsi and Gares varieties were less suitable pomegranate varieties for both *E. ceratoniae* and *D. livia*. Consequently, Guebsi was designated as the least suitable cultivar for *E. ceratoniae*; Gares was the least suitable

cultivar for *D. livia*. To prevent or reduce infestation of pomegranates by these pests, we recommend growing these cultivars in locations where damage by *E. ceratoniae* and *D. livia* is typically high. Further studies on the competition between the two pests under field conditions are needed.

**Acknowledgments.** This research was supported by the Regional Research Center in Oasis Agriculture of Degache, Tunisia. This research was funded by Taif University, Saudi Arabia through project number (TU-DSPP-2024-158).

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