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# Promising management of two destructive insect pests of pomegranates (*Punicagranatum*L.) in Tunisia through the optimization of harvesting time †

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**Abstract:** This work was set up to characterize the incidence of *Ectomyelois ceratoniae* and *Virachola livia*, to link infestation with pomegranates maturity and insects' densities and to identify potential resistant/tolerant varieties. Three Tunisian pomegranate cultivars (Tounsi, Gebsi,Gares) grown under oasian conditions were employed. Fruit and juice chemical compositions were determined from green fruit to maturity. Moreover, fruit infestation rates were assessed at each stage. Results indicated that infestation increased as fruits ripened. Results pointed out that pomegranate infestation was related to changes in fruit composition mainly titrable acidity, total soluble solids and total phenolics and flavonoids contents.

**Keywords:** *Ectomyelois ceratoniae*; *Virachola livia*; Pomegranate; Fruit ripeness; Infestations; Oasis.

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## 1. Introduction

Pomegranate *Punica granatum* has been cultivated and naturalized over the whole Mediterranean region since antiquity. In Tunisia, pomegranate has been cultivated traditionally under diverse agro-climatic conditions. It is well known typically in the coastal regions and in many regions inside the country [1]. It is also considered us a basic crop in the oases [2].

Several studies shows also the nutritional and medicinal benefits of pomegranate, such as antioxidant [3],antiallergic[4], antimicrobial [5] andantidiabetic[6]. Since ancient time, pomegranate has been used as a natural astringent for treating diarrhea and harmful internal parasites [7]. Its fruit contains considerable amount of protein, sugar and mineral [8].

Indeed pomegranate cultivation is affected by a number of problems such as fruit cracking, fruit rot and the attack of many pests. Two major insect pests species such the carob moth *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) recognized as one of the most destructive insect pests of pomegranate caused loss of weight and downgrading of the commercial value of fruits [9]. Since 2006, severe unusual damages detected on pomegranates, which had never been observed in the Tunisian microclimate, were attributed to a new invasive insect pest [10]. The study of the morphology of this pest led to its identification as *Virachola livia* Klug 1834 (Lepidoptera: Lycaenidae).

However, since the use of resistant varieties is a key tool in supporting a successful integrated pest management program. The evaluation of the resistance of various culti-

vars to pests may offer useful information about their attraction or not for the target pest species [11].

The aim of the present investigation was to examine whether the variation of chemical properties of pomegranate during maturation can affect the infestation rate of those pests and to identify potential resistant/tolerant varieties. To this end, we quantified some pomegranate compounds and properties such as total phenolics and flavonoids contents, pH, total soluble solids content, and titrable acidity, and determined a relationship between their levels variation and the infestation of those insect pests.

Our findings will inform a novel *E. ceratoniae* and *V. livia* management approaches, including the use of resistant cultivars to reduce the damage caused by those pests in orchard systems.

## 2. Materials and Methods

### *2.1. Plant materials*

Three pomegranate cultivars grown under oasian conditions in Tozeur region (South-western Tunisia) were studied: Gebsi, Tounsi and Gares.

Fresh pomegranate fruits (*Punica granatum* L.) of each cultivar were selected. Fruits were picked manually at different stages after fruit set. A number of nine fruits from each cultivars, which are classified into three lots, were taken different time during maturity based on the subject evaluation on skin color.

### *2.2. Infestation rate*

Fruit sampled from each variety were randomly taken, dissected and examined in the laboratory to evaluate the infestation rates of *E. ceratoniae* and *V. livia*. The infested and non-infested fruits registered individually and the infestation rate is calculated using the following equation:

$$\text{Infestation rate (\%)} = \frac{\text{Infested fruit number}}{\text{Examined total fruit number}} \times 100$$

### *2.3. Titrable acidity, pH, total soluble solids content*

Titrable acidity (TA) was determined by titration to pH 8.1 with 0.1M NaOH solution and expressed as g of citric acid per 100 ml of juice. The pH was measured using a digital pH meter.

The total soluble solids, TSS, (indicate the level of sugars) were determined with a digital refractometer (Erma, Tokyo, calibrated using distellid water) and the results were reported as degree Brix at 21°C. Index of maturity (IM) can be also calculated by devising the TSS contents to TA.

### *2.4. Total phenolic content*

Total phenolics weredetermined using Folin-Ciocalteu method. The result was expressed as milligram of Gallic acid equivalent per one milliliter of pomegranate juice.

### *2.5. Flavonoid content Determination*

Total flavonoid content was measured by Aluminum chloride colorimetric assay. It was expressed as milligram rutine equivalent per one milliliter of pomegranate juice.

## 3. Results

### *3.1. Infestation rate*

The analysis of the infestation rate is shown in Figure 1.

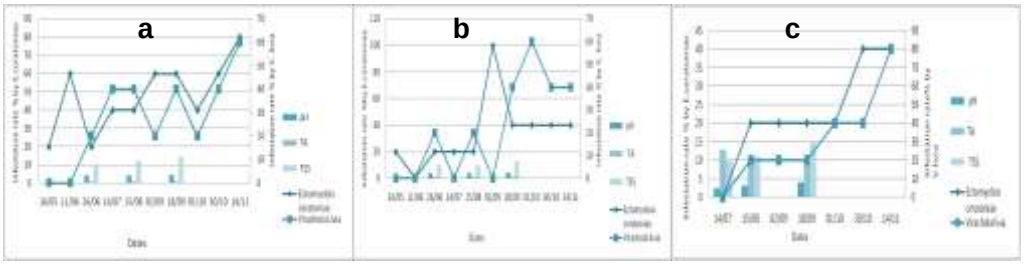


**Figure 1.**Variation of the infestation rates of pomegranate fruits by both *E.ceratoniae* and *V. livia*: (**a**) Infestation rate of the variety Tounsi; (**b**) Infestation rate of the variety Gebsi;(**c**) Infestation rate of the variety Gares.

The results pointed out that *E. ceratoniae* and *V. livia* reach their maximum of infestation with advance in maturity of pomegranate fruit. At the green stage, respectively 20% and 0% of pomegranates from Tounsi and Gebsi cultivar and 0% from Gares cultivar were infested by *E. ceratoniae* and *V. livia*. Then, at ripe stage, infestation reached 80% for both *E. ceratoniae* and *V. livia*.

For all pomegranate cultivars the infestation by *E. ceratoniae* was earlier than the infestation by *V. livia* which is began few weeks after the appearance of *E. ceratoniae* in orchards.

*3.2. Chemical properties*

The results for the chemical properties of the pomegranate cultivars in this research demonstrate that pH of pomegranate juice increased with ripening, being a maximum 3.94, 4.13 and 4.51 respectively for Gares, Gebsi and Tounsi at the maturity. The titrable acidity decreased with the advance in maturity. This study showed that mature fruits were less acidic (0.88, 0.57 and 10.26 for Gebsi, Tounsi and Gares respectively) than immature ones (0.99, 1.02 and 12.71 respectively for Gebsi, Tounsi and Gares )(Figure2).

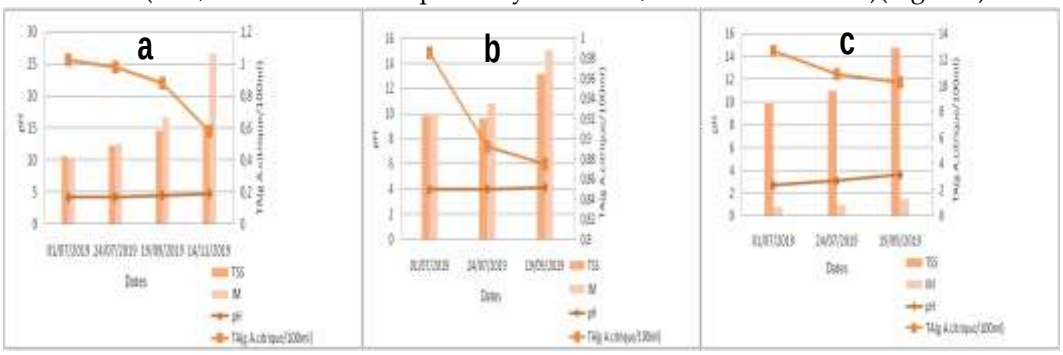


**Figure 2.** Variation of chemical properties mesured during maturity for pomegranate cultivars: (**a**)Tounsi; (**b**) Gebsi and (**c**) Gares.

Values of the soluble solids (Brix index) content increased significantly with advance in maturity, being 9.8, 10.56 and 9.86 for immature fruits of Gebsi , Tounsi and Gares respectively, and 13.16, 15.26 and 14.76 for mature fruits of Gebsi , Tounsi and Gares respectively.

Most notable difference between those cultivars were detected in the titrable acidity value wich is higher in Gares during ripening than Tounsi and Gebsi.

The total phenolics and flavonoid analysis results for the pomegranate cultivars investigated are presented in Figure 3.

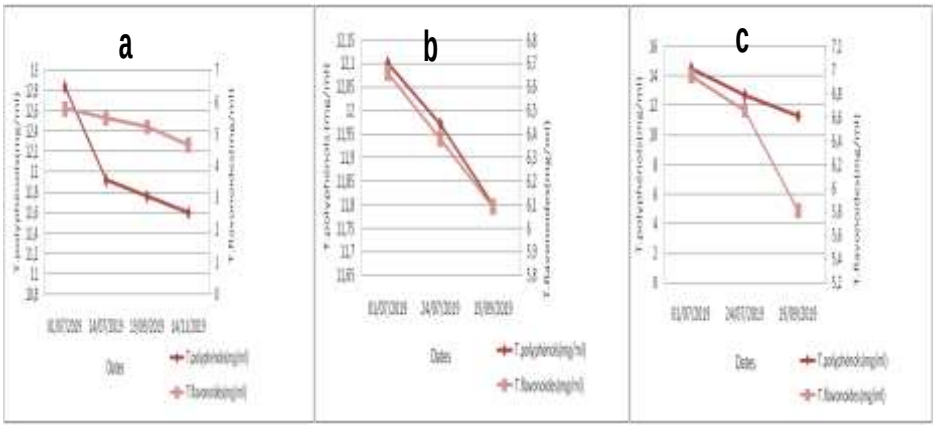


**Figure 3.**Variation of the total polyphenols and flavonoids contents during maturity of pomegranate cultivars examined: (**a**)Variation of the total polyphenols and flavonoids contents of the variety Tounsi; (**b**) Variation of the total polyphenols and flavonoids contents of the variety Gebsi; (**c**) Variation of the total polyphenols and flavonoids contents of the variety Gares.

The highest level of total phenolics was observed in Gares (14.51 mg/ml) and the lowest one in Tounsi (12.84 mg/ml) and Gebsi (12.1 mg/ml).values of phenolics were decreased with maturity being 11.32, 11.8 and 11.6 for Gares, Gebsi and Tounsi respectively.Flavonoid analysis results showed that the less level of flavonoid content were observed at the maturity of fruits with 5.81, 6.09 and 4.67 for Gares, Gebsi and Tounsi respectively.

## 4. Discussion

Research on plant-herbivore interactions is one of the most important and multidisciplinary undertakings in plant biology, involving various disciplines to describe chemical and ecological process influencing the outcome of plant-herbivore interactions [12].

The results of the present study indicated that *E. ceratoniae* and *V. livia* are able to infest all pomegranate cultivars. This infestation increased with advance in maturity of fruits. The less insect attacks in those varieties, at green fruit immature, may be attributed to the amount of acid content of this variety as reported by [13].

In the present research, infestation rate of pomegranate cultivars is higher at the maturity of fruit, apparently as a consequence of the lower totals polyphenol, flavonoid contents, titrable acidity and the higher TSS content (1 Brix corresponding to 1 g sucre in 100 g of solution).

Our results indicate that the variety Gebsi is less infested by *E. ceratoniae* and *V. livia* at the maturity which is agreement with [10], who reported that levels of *V. livia* infection were shown to vary among pomegranate varieties and that the variety Gebsi was more tolerant to this lycaenidae.

Results put out that the TA was still higher with ripening in the variety Gares than Tounsi and Gebsi. Whereas the total soluble solids (TSS) content was increased and comparable on pomegranate cultivars investigated during maturity.

These findings can approve that the high infestation rate of pomegranate fruits (*Punica granatum* L.) by *E. ceratoniae* and *V. livia* is mainly due to the higher TSS contents.

## 5. Conclusion

This research demonstrated the existence of interactions between chemical characteristics of various pomegranate cultivars with infestation rate of those pests.

Gebsi cultivar was the less infested by both pests. This study showed that under oasian conditions, the management of *E. ceratoniae* and *V. livia* population could be achieved through the use of resistant/tolerant pomegranate cultivars. Indeed, for a sus-

tainable agricultural production under the oasian fragile ecosystem, the use of such cultivars may play a crucial role in protecting the environment and reducing losses of pests.

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