

## Effect of Chemical Characteristics of Pomegranate Varieties on the Infestation of Pomegranate Fruit Moth

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### ABSTRACT

This study was conducted to investigate the susceptibility of three local pomegranate varieties (Salimi, Khadrawi, and Shukar) to infestation by the pomegranate fruit worm *Ectomyelois ceratoniae* during different fruit maturity stages, as well as to identify some chemical characteristics of these varieties and their role in the infestation process. The results revealed variations in infestation percentage among the studied varieties, with Shukar being the most susceptible (50.18% infestation rate) and Salimi the least susceptible (35.47%). Infestation percentage also varied by maturation stage, with ripe fruits showing the highest infestation (72.11%) and unripe fruits the lowest (25.44%). Chemical analysis revealed a positive correlation between infestation percentage and carbohydrate content, while total phenols, tannins, and protein content showed a negative correlation with infestation as the fruits matured.

**Key words:** carbohydrate, maturation, phenolics, protein, stages, tannin.



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**Received: 11/4/2025, Accepted: 9/7/2025, Published: 31/7/2026**

### INTRODUCTION

The pomegranate (*Punica granatum* L.) is an economically important fruit tree, ranking highly among Arab countries, with Iraq cultivating approximately 5,449,000 trees and producing 234,292 tons during the summer season. Diyala Governorate led production at 53.06% of Iraq's total output, followed by Salahuddin, while Karbala ranked third at 3.64% (Central statistical organization, 2021). Pomegranate trees are grown in many regions worldwide, including the Mediterranean (Kandyli and Kokkinomagoulos, 2020). Many studies highlight its nutritional and therapeutic benefits due to its antioxidants (Elfalleh et al., 2009), antiallergens (Damiani et al., 2009), antimicrobial characteristics, anti-inflammatory effects and industrial applications (Al-Zoreky, 2009). The value of the fruit is due mainly to its antioxidant action, and its secondary metabolites, such as punicalagin, anthocyanins, phenolics and tannins (Abedi et al., 2020). Pomegranate

cultivation is susceptible to several biotic and abiotic factors like fruit cracking, rot and pest infestations (Ksentini et al., 2011). The pomegranate fruit moth *E. ceratoniae* is a key pest, presenting up to 80% yield loss depending on location and season (Mamay et al., 2014; Abdel-Galil et al., 2023) and an economical damage has been reported in pomegranate and date palm orchards of U.S., Iran, Iraq, Tunisia and Morocco (Abid et al., 2021). The objective of this study was to investigate the role of some chemical traits of pomegranate fruits throughout different ripening stages in conferring resistance to infestation by the pomegranate fruit moth *Ectomyelois ceratoniae*.

### MATERIALS AND METHODS

The study was conducted at a pomegranate orchard planted with three varieties (Salimi, Khadrawi, and Shukar) located in Al-Husseiniya District, Karbala, during the 2024 season. The orchard spanned 15 dunams, with 400 pomegranate trees (representing the

three varieties). No insecticide treatments were applied during the study period. Homogeneous trees aged 20–25 years were selected for sampling. Sampling was carried out weekly throughout the 2024 season, starting from the fruit formation stage in May until harvest time in November. Five pomegranate trees were randomly selected from each cultivar. Each week, 50 fruits were collected per cultivar (10 fruits per tree), ensuring that samples were taken from different locations representing the four cardinal directions of the plant. This resulted in a total sample size of 150 fruits per sampling event. The collected fruits were transported to the laboratory, dissected, and examined under a light microscope for signs of infestation. Infestation percentage were recorded based on the presence of larvae, pupae, and visible damage symptoms. The percentage of infestation was calculated using the formula proposed by (Silvie and Gozé, 1991):

$$\text{Infestation Percentage (\%)} = \frac{\text{Number of Infested Fruits}}{\text{Total Number of Fruits Sampled}} \times 100$$

Three pomegranate trees were selected from each of the studied varieties (Sulaimi, Khudrawi, and Shukar). A total of nine randomly selected fruits were collected at each of three ripening stages: unripe, semi-ripe, and fully ripe (Cirillo et al., 2022; Hamad et al., 2024). The fruits were collected on June 5, August 5, and October 5, 2024. Only healthy, undamaged fruits were chosen, labeled by cultivar, placed in appropriate bags, and transported to the laboratory of the Ministry of Science and Technology for analysis. The estimation of phenols was conducted according to Arnow's method (1955), and the absorbance was measured at a wavelength of 515 nm using a spectrophotometer for the colored complex resulting from specific reactions of Arnow's Reagent with ortho-dihydroxy phenols. The samples were prepared as follows:

#### **Preparation of the alcoholic extract:**

1 g of the tissue (sample) was taken and 10 ml of 80% ethyl alcohol was added. The reaction was heated at 100 °C in a water bath for 10 min, and then placed in a cold water bath. The sample was pulverized with a porcelain mortar

and filtered using two layers of gauze fabric. The residue was re-extracted with 5 ml of ethyl alcohol and again filtered using gauze fabric. These filtrate percentage were pooled and filtered again using Whatman No. 4. The volume was adjusted with ethyl alcohol to a final volume of 10 ml per 1 g of fresh tissue weight, and this extract was used for phenol estimation.

#### **Preparation of Arnow's reagent**

10 grams each of sodium nitrite ( $\text{NaNO}_2$ ) and sodium molybdate ( $\text{Na}_2\text{MoO}_4$ ) were dissolved in 100 ml of distilled water and kept in a sterile glass bottle until use.

#### **Total phenolics determination**

A volume of 1 ml of the alcoholic extract was transferred into a test tube. Sequential addition of 1 ml of 0.05 N hydrochloric acid (HCl), 1 ml of Arnow's reagent, 10 ml of distilled water, and 2 ml of sodium hydroxide (NaOH) were made to this. The absorbance of the formed color complex was read at 515 nm with a spectrophotometer. Standard calibrating curve was prepared with catechol ( $\text{C}_6\text{H}_4(\text{OH})_2$ ) to calculate total phenolic content.

Total tannin concentration was calculated as previously established by Dewi et al. (2014). The 0.05 g sample of pomegranate tissue (peel, pulp, juice) was boiled with 80 ml of distilled water for an hour. The cooled mixture was filtered and then the volume of the filtrate was made up to 100 ml with distilled water.

#### **Preparation of tannic acid standard Curve:**

-A set of 25 ml volumetric flasks containing a range of standard tannic acid solutions with final concentrations ranging from 1 to 6  $\mu\text{g/ml}$  were prepared.

-Each flask received 2.5 ml of ferric ion solution ( $\text{Fe}^{3+}$ ) and the mixtures were maintained at 80°C using a water bath for 20 minutes.

-Pipetted into each of the flasks 2.5 ml of acetate buffer, 0.5 ml of 1,10-phenanthroline solution, and 0.5 ml of ethylene diamine tetraacetic acid (EDTA) solution adjust to pH 4.4. We then injected a mixture of reagents.

-The flasks were cooled to room temperature.

-Absorbance was recorded at 508 nm by a spectrophotometer.

The Kjeldahl method (1954) was used to determine the protein content. 1g portion was

transferred into a digestion tube, then 1 g of catalyst copper sulfate ( $\text{CuSO}_4$ ), as well as 5 ml 98% concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$ ) was added. The digestion tubes were heated until a clear solution formed and then allowed to cool. Then, 25 ml of distilled water and 10 ml of sodium hydroxide ( $\text{NaOH}$ ) was added to the digested mixture. The final reaction solution was evaporated and the ammonia gas released was absorbed by 25 ml of 2% boric acid solution. Then the volume of ammonia was titrated with 0.01 N formation hydrochloric acid ( $\text{HCl}$ ) according to the method described by AOAC (1980) and the percentage of protein was calculated using the formula given below:

**% Protein** = (Volume of  $\text{HCl}$  used  $\times$  Normality of  $\text{HCl} \times 14.007 \times 6.25$ ) / Sample weight (g)

Where: 14.007 is the molecular weight of nitrogen  
6.25 is the nitrogen-to-protein conversion factor

Sample weight is in grams

This method provides an accurate estimate of the total crude protein content in the pomegranate samples analyzed.

Total carbohydrate was determined by the method of Joslyn (1970). 0.2 g portion was combined with 8.8 ml 80% ethanol and kept in a water bath at 60 °C for 30 min. afterward, the extract was centrifuged at 3,000 rpm for 15 min. The residue was re-extracted using 10 ml of 80% ethanol, followed by 2 further rounds of centrifugation under the same conditions. Supernatants were pooled and brought to 50 ml with 80% ethanol. The extract was transferred to the test tube 1ml and phenol solution 5% 1ml was added and 5ml concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ). The absorbance was measured using a spectrophotometer at 490 nm after cooling. A standard curve was established with glucose solutions of known concentrations: 0, 0.5, 1, 1.5, 2, 2.5, and 3g of glucose dissolved in a 100 ml of distilled water. As for each concentration, 1 ml of the solution and 1 ml of 5% phenol and 5 ml concentrated sulfuric acid were applied according to the above-mentioned procedure. Absorbance values were determined at these respective concentrations and used to create a glucose standard curve. The experiment was designed according to the Randomized Complete Block Design (RCBD)

to assess the sensitivity of the varieties of pomegranate for infection with fruit moth. The results were analyzed using the analysis of the variance (the ANOVA) table. The means were compared using least significant difference (LSD) at a significance level of 0.05. The statistical software SAS was used for data analysis (Antar, 2010).

## RESULTS AND DISCUSSION

### Percentage of pomegranate fruit infestation by *Ectomyelois ceratoniae*:

The results in (Table 1). show that during the unripe stage, the highest infestation percentage by *Ectomyelois ceratoniae* was recorded on the Shukar cultivar at 12.18%, while the lowest was observed on Salimi at 7.79%. In the semi-ripe stage, Shukar also exhibited the highest infestation rate (44.39%), whereas Salimi showed the lowest (26.76%). During the fully ripe stage, Shukar again had the highest infestation percentage at 74.54%, while Salimi recorded the lowest at 56.23%. Analysis of variance (ANOVA) showed that the differences among the varieties were highly significant. Overall, the Salimi cultivar exhibited the lowest infestation rate (31.94%), with Khadrawi and Shukar having higher (38.43%) and the highest (46.14%) infestation percentage, correspondingly. There were also significant differences between fruit maturity stages. Ripe stage gave the highest (64.13%) and unripe stage the lowest (9.81%) mean infestation rate. Immature fruits typically have increased levels of defensive metabolites, like phenolics and tannins, making the fruit less palatable to the insect and more difficult to invade. Braham (2015) reported similar findings in a study of nine pomegranate varieties, noting that all were susceptible to *E. ceratoniae* larvae except for one cultivar considered resistant to lepidopteran pests. Similarly, Moawad et al. (2011) reported that the Wonderful variety is resistant to *E. ceratoniae* infestation. These results are consistent with the findings of Hamad et al. (2024), who reported that the infestation rate by *E. ceratoniae* and *D. livia* in the Gafsa and Degache oases in Tunisia peaked as pomegranate fruits reached maturity.

**Table 1.** Percentage of infestation by the pomegranate fruit moth *Ectomyelois ceratoniae* according to fruit ripening stages in different pomegranate varieties

| Varieties       | Percentage of Infestation % |           |                | Mean varieties |
|-----------------|-----------------------------|-----------|----------------|----------------|
|                 | Unripe Stage (%)            | Semi-Ripe | Ripe Stage (%) |                |
| Sulaimi         | 7.79                        | 26.76     | 56.23          | 31.94          |
| Khudrawi        | 9.46                        | 37.93     | 61.63          | 38.43          |
| Shukar          | 12.18                       | 44.39     | 74.54          | 46.14          |
| Mean ripe stage | 9.81                        | 36.36     | 64.13          |                |
| L.S.D5%         | Ripe stage                  | Varieties | Interaction    |                |
|                 | 3.170                       | 3.089     | 5.491          |                |

**Effect of phenolic content in pomegranate fruits:** The results showed variations in phenolic content during ripening stages and varieties (Table 2). The highest phenolic content was recorded in the unripe stage of the Salimi cultivar in the peel tissue, reaching 725.3 mg/g, while the lowest was observed in the Shukar cultivar at 511 mg/g. During the semi-ripe stage, Salimi again showed the highest phenolic content (693.7 mg/g), whereas Shukar had the lowest (479 mg/g). In the ripe stage, the highest phenolic concentration was also found in Salimi (590.3 mg/g), with the lowest in Shukar (367.3 mg/g). The results further indicate significant differences among ripening stages. A

higher average phenolic value (593.3 mg/g) was found in the unripe stage, while the lower value (468.4 mg/g) was detected in ripe stage. A significant variation was also observed among varieties. The determined mean phenolic content of the cultivar (Salimi) was 669.8 mg/g (maximum) while the minimum value was measured at 452.4 mg/g in the case of Shukar cultivar. The degree infestation of fruit peels at various ripening levels showed a strong negative correlation with their total phenolic content, which suggests a relationship between higher phenolic concentrations in fruit peel and lower levels of infestation (Braham, 2015).

**Table 2.** Phenolic content in pomegranate fruit peel (mg/g)

| Varieties       | Phenolic content across pomegranate fruit ripening stages (mg/g) |           |                | Mean varieties  |
|-----------------|------------------------------------------------------------------|-----------|----------------|-----------------|
|                 | Unripe Stage (%)                                                 | Semi-Ripe | Ripe Stage (%) |                 |
| Sulaimi         | 725.3                                                            | 693.7     | 590.3          | 669.8           |
| Khudrawi        | 543.7                                                            | 494.3     | 447.7          | 495.2           |
| Shukar          | 511                                                              | 479       | 367.3          | 452.4           |
| Mean ripe stage | 593.3                                                            | 555.7     | 468.4          |                 |
| L.S.D5%         | Ripe stage                                                       | Varieties | Interaction    | Correlation = - |
|                 | 35.28                                                            | 35.28     | 61.11          |                 |

The results in (Table 3). reveal significant differences in phenolic values between different varieties and fruit ripening stages. In the pulp of the Salimi cultivar recorded the highest phenolic content (488.67 mg/g) occurred in the unripe stage, while the lowest; in the semi-ripe stage in the Shukar cultivar (429.33 mg/g). Salimi had the highest phenol content (485.33 mg/g), and Shukar the lowest (425.67 mg/g). In the ripe stage, the maximum phenolic in Salimi (395.33 mg/g) and the minimum in Shukar (322.33 mg/g) were recorded. The findings revealed differences among stages of ripening, too. Mean phenolic content was highest at the unripe stage (458.67 mg/g) and lowest at the ripe stage (364.11 mg/g). Significant differences were observed

among varieties, with Salimi cultivar having the highest mean phenolic content in pulp (456.44 mg/g) and Shukar cultivar having the lowest (392.44 mg/g). A very strong, negative correlation was found between the infestation percentage and total phenolic content in the pulp, which might indicate that an increase in phenolics leads to a lower susceptibility to infestation of the fruit, the decreased concentration of defensive phenolic compounds during pomegranate fruit ripening acts as a major factor that increases fruit susceptibility to pests, explaining the strong and statistically significant negative correlation between the two variables during fruit development stages (Dewi et al., 2014).

**Table 3.** Phenolic content in pomegranate fruit pulp (mg/g)

| Varieties       | Phenolic content across pomegranate fruit ripening stages (mg/g) |                     |                | Mean varieties         |
|-----------------|------------------------------------------------------------------|---------------------|----------------|------------------------|
|                 | Unripe Stage (%)                                                 | Semi-Ripe Stage (%) | Ripe Stage (%) |                        |
| Sulaimi         | 488.67                                                           | 485.33              | 395.33         | 456.44                 |
| Khudrawi        | 458.00                                                           | 449.33              | 374.67         | 427.33                 |
| Shukar          | 429.33                                                           | 425.67              | 322.33         | 392.44                 |
| Mean ripe stage | 458.67                                                           | 453.44              | 364.11         |                        |
| L.S.D5%         | Ripe stage                                                       | Varieties           | Interaction    | Correlation = - 0.63** |
|                 | 3.972                                                            | 3.972               | 6.880          |                        |

(Table 4). shows variation in phenolic content across varieties and ripening stages. The highest phenolic concentration in the juice was found at the unripe stage for Salimi cultivar (191.33 mg/g) but a lower compound was recorded in Shukar cultivar (172.00 mg/g). In semi ripe stage again Salimi cultivar had the highest phenolic content (175.67 mg/g), while the lowest was found in Shukar cultivar (153.00 mg/g). In the ripe stage, Salimi had the highest content (145.33 mg/g), whereas the lowest was again in Shukar at 101.00 mg/g. The results also revealed significant differences between ripening stages. The mean

phenolic content in the unripe stage represented the highest value (183.00 mg/g), the mean phenolic content in the ripe stage represented the lowest value (124.00 mg/g). Also, cultivar effects were highly significant. The maximum (170.78 mg/g) and the minimum (142.00 mg/g) average phenolic content in juice belonged to Salimi and Shukar cultivar respectively. The negative correlations observed between infestation percentage and total phenolic content in the juice during fruit ripening stages were highly significant (Dehghanian et al., 2022).

**Table 4.** Phenolic content in pomegranate juice (mg/g)

| Varieties       | Phenolic content across pomegranate fruit ripening stages (mg/g) |                     |                | Mean varieties  |
|-----------------|------------------------------------------------------------------|---------------------|----------------|-----------------|
|                 | Unripe Stage (%)                                                 | Semi-Ripe Stage (%) | Ripe Stage (%) |                 |
| Sulaimi         | 191.33                                                           | 175.67              | 145.33         | 170.78          |
| Khudrawi        | 185.67                                                           | 155.00              | 125.67         | 155.44          |
| Shukar          | 172.00                                                           | 153.00              | 101.00         | 142.00          |
| Mean ripe stage | 183.00                                                           | 161.22              | 124.00         |                 |
| L.S.D5%         | Ripe stage                                                       | Varieties           | Interaction    | Correlation = - |
|                 | 4.223                                                            | 4.223               | 7.314          |                 |

Torshiz et al. (2020) indicated the presence of an inverse relationship between infestation by *E. ceratoniae* and total phenolic content. The total phenolic content in pomegranate juice showed negative correlations with infestation by the pomegranate fruit moth. Similarly, Hamad et al. (2024) demonstrated that *E. ceratoniae* can infest all pomegranate varieties, with infestation percentage increasing as the fruits mature. They found that the Tunisian Gares pomegranate variety was less susceptible to *E. ceratoniae* infestation during the unripe stage. This reduced susceptibility may be attributed to lower pH values and higher polyphenol content compared to the ripe stage. It is well known that plants employ various volatile

compounds as advanced chemical languages for communication and defense against pest damage (Hamad et al., 2024). Multiple studies confirm that pomegranate peels contain the highest polyphenol content surpassing seeds and juice (Kandyliis and E. Kokkinomagoulos, 2020).

#### **Tannin percentage in pomegranate fruits**

The results illust percentage the variation in tannin content across varieties and ripening stages (Table 5). The highest tannin concentration was observed in the unripe stage of the Salimi cultivar in the peel, reaching 22.197%, while the lowest was recorded in the Shukar cultivar at 18.453%. In the semi-ripe stage, Salimi again had the highest tannin content (20.470%), whereas Shukar had the

lowest (18.747%). During the ripe stage, Salimi showed the highest tannin concentration at 19.847%, while Shukar recorded the lowest at 17.920%. Results also showed difference between ripening stages. While the unripe stage had the highest average tannin content (19.888%) and the ripe stage had the lowest average tannin content (18.766%). Moreover, significant differences were noticed in varieties. The highest

percentage of tannin was found in the Salimi cultivar (20.838%) followed by Shukar with a lowest (18.373%). There was noted a highly significant negative correlation between the percentage of infestation and tannin content in the fruit peel at ripening stages: an increase in tannin content results in a decrease in infestation of *Ectomyelois ceratoniae* (Fotouhi et al., 2024).

**Table 5.** Tannin percentage in pomegranate fruit peel (%)

| Varieties       | Tannin content across pomegranate fruit ripening stages (%) |           |                | Mean varieties  |
|-----------------|-------------------------------------------------------------|-----------|----------------|-----------------|
|                 | Unripe Stage (%)                                            | Semi-Ripe | Ripe Stage (%) |                 |
| Sulaimi         | 22.197                                                      | 20.470    | 19.847         | 20.838          |
| Khudrawi        | 19.013                                                      | 18.683    | 18.530         | 18.742          |
| Shukar          | 18.453                                                      | 18.747    | 17.920         | 18.373          |
| Mean ripe stage | 19.888                                                      | 19.300    | 18.766         |                 |
| L.S.D5%         | Ripe stage                                                  | Varieties | Interaction    | Correlation = - |
|                 | 0.3561                                                      | 0.3561    | 0.6167         |                 |

#### Tannin percentage in pomegranate pulp

(Table 6). indicates variation in tannin content across ripening stages and varieties. The highest tannin content in the pulp was recorded in the unripe stage of the Salimi cultivar at 18.120%, while the lowest was observed in the Shukar cultivar at 17.400%. In the semi-ripe stage, Salimi again had the highest tannin level (17.310%), and Shukar had the lowest (16.400%). During the ripe stage, Salimi maintained the highest content at 17.227%, while Shukar recorded the lowest at 16.037%. Statistical analysis confirmed significant maturation-stage effects ( $p < 0.05$ ), with tannin content progressively declining from unripe (17.748%) to ripe stages (16.802%). A cultivar difference was also highly significant. Salimi cultivar possessed

the highest pulp tannin mean content (17.552%), whilst Shukar cultivar showed the least amount (16.612%), according to the results. A highly significant negative correlation was noted between infestation percentage and tannin content in the pulp through the ripening stages, revealing that higher levels of tannins may provide better resistance to the attack of *Ectomyelois ceratoniae* infestation, , the decreased concentration of defensive tannin during pomegranate fruit ripening acts as a major factor that increases fruit susceptibility to pests, explaining the strong and statistically significant negative correlation between the two variables during fruit development stages (Kavianpour et al., 2014).

**Table 6.** Tannin percentage in pomegranate pulp (%)

| Varieties       | Tannin content across pomegranate fruit ripening stages (%) |           |                | Mean varieties  |
|-----------------|-------------------------------------------------------------|-----------|----------------|-----------------|
|                 | Unripe Stage (%)                                            | Semi-Ripe | Ripe Stage (%) |                 |
| Sulaimi         | 18.120                                                      | 17.310    | 17.227         | 17.552          |
| Khudrawi        | 17.723                                                      | 16.860    | 17.143         | 17.242          |
| Shukar          | 17.400                                                      | 16.400    | 16.037         | 16.612          |
| Mean ripe stage | 17.748                                                      | 16.857    | 16.802         |                 |
| L.S.D5%         | Ripe stage                                                  | Varieties | Interaction    | Correlation = - |
|                 | 0.1062                                                      | 0.1062    | 0.1840         |                 |

(Table 7). shows variation in tannin content in the juice across different varieties and fruit ripening stages. The highest tannin content was recorded in the unripe stage of the Salimi cultivar at 1.877%, while the lowest was found

in the Shukar cultivar at 1.823%. During the semi-ripe stage, Salimi again showed the highest tannin level (1.843%), whereas Shukar had the lowest (1.730%). In the ripe stage, the highest content was still found in Salimi

(1.197%), and the lowest was recorded in Shukar at 0.870%. Statistical analysis confirmed significant ( $p < 0.05$ ) between ripening stages, with tannins declining sharply from unripe (1.856%) to ripe stages (1.022%). In addition, significant differences between varieties were recognized. The juice of Salimi cultivar had the highest tannin content (1.639%) followed by other varieties, and in

contrast to Shukar (1.474%) which had the least. The strong negative relationship found between infestation percentage and juice tannins content at each of the studied ripening stages implied that juice of fruits with higher tannin levels probably accounting for lower *Ectomyelois ceratoniae* susceptibility (Rat and Mamay, 2024).

**Table 7.** Tannin percentage in pomegranate juice (%)

| Varieties       | Tannin content across pomegranate fruit ripening stages (%) |           |                | Mean varieties  |
|-----------------|-------------------------------------------------------------|-----------|----------------|-----------------|
|                 | Unripe Stage (%)                                            | Semi-Ripe | Ripe Stage (%) |                 |
| Sulaimi         | 1.877                                                       | 1.843     | 1.197          | 1.639           |
| Khudrawi        | 1.867                                                       | 1.803     | 1.000          | 1.557           |
| Shukar          | 1.823                                                       | 1.730     | 0.870          | 1.474           |
| Mean ripe stage | 1.856                                                       | 1.792     | 1.022          |                 |
| L.S.D5%         | Ripe stage                                                  | Varieties | Interaction    | Correlation = - |
|                 | 0.0703                                                      | 0.0703    | 0.1218         |                 |

Tannins are well known for their diverse roles in stimulating plant defense responses and protecting plants from various stressors, particularly biotic stress. Elevated tannin levels in different plant organs contribute to bitterness, which enhances resistance to herbivorous insects (Dehghanian et al. 2022). The pomegranate peel is particularly rich in acidic compounds, including tannins, with concentrations ranging from 20–25%, these tannins belong to the gallotannin group, which includes compounds such as Punicalin Carana, Punicalagin, and Ellagic Acid (Gil, 2000).

#### Protein percentage in pomegranate fruits

(Table 8). shows variation in protein content across ripening stages and varieties. The highest protein content in the peel was recorded during the unripe stage of the Salimi cultivar at 4.177%, while the lowest was in the Shukar cultivar at 4.030%. In the semi-ripe stage, Salimi again showed the highest protein level (3.897%), whereas Shukar recorded the

lowest was (3.727%). During the ripe stage, the highest protein content was still observed in Salimi (3.817%), and the lowest in Shukar at 2.593%. The results also revealed significant differences among ripening stages. The unripe stage showed the highest average protein content at 4.119%, while the lowest was recorded in the ripe stage at 3.317%. Furthermore, significant differences were found among varieties. Significant differences were also observed among the varieties. The Sulaimi cultivar achieved the highest average protein content at 3.963%, while the Shakar cultivar had the lowest average content at 3.450%. Furthermore, a negative correlation was observed between the percentage of infestation by *E. ceratoniae* and protein content in the fruit peel across different maturity stages. This suggests that higher protein levels may contribute to reduced pest infestation percentage, potentially playing a role in plant defense mechanisms.

**Table 8.** Protein percentage in pomegranate fruit peel (%)

| Varieties       | Protein content across pomegranate fruit ripening stages (%) |           |                | Mean varieties  |
|-----------------|--------------------------------------------------------------|-----------|----------------|-----------------|
|                 | Unripe Stage (%)                                             | Semi-Ripe | Ripe Stage (%) |                 |
| Sulaimi         | 4.177                                                        | 3.897     | 3.817          | 3.963           |
| Khudrawi        | 4.150                                                        | 3.877     | 3.540          | 3.856           |
| Shukar          | 4.030                                                        | 3.727     | 2.593          | 3.450           |
| Mean ripe stage | 4.119                                                        | 3.833     | 3.317          |                 |
| L.S.D5%         | Ripe stage                                                   | Varieties | Interaction    | Correlation = - |
|                 | 0.363                                                        | N.S       | 0.628          |                 |

(Table 9). shows variation in protein content during fruit ripening stages and varieties. The highest protein content in the pulp was recorded during the unripe stage of the Salimi cultivar at 2.683%, while the lowest was observed in the Shukar cultivar at 2.440%. In the semi-ripe stage, Salimi again exhibited the highest protein level (2.550%), and Shukar the lowest (2.387%). During the ripe stage, Salimi maintained the highest protein content (2.383%), whereas the lowest was recorded in Shukar at 2.100%. The results also indicated significant differences among ripening stages.

**Table 9.** Protein percentage in pomegranate pulp (%)

| Varieties       | Protein content across pomegranate fruit ripening stages (%) |           |                | Mean varieties  |
|-----------------|--------------------------------------------------------------|-----------|----------------|-----------------|
|                 | Unripe Stage (%)                                             | Semi-Ripe | Ripe Stage (%) |                 |
| Sulaimi         | 2.683                                                        | 2.550     | 2.383          | 2.539           |
| Khudrawi        | 2.547                                                        | 2.373     | 2.417          | 2.446           |
| Shukar          | 2.440                                                        | 2.387     | 2.100          | 2.309           |
| Mean ripe stage | 2.557                                                        | 2.437     | 2.300          |                 |
| L.S.D5%         | Ripe stage                                                   | Varieties | Interaction    | Correlation = - |
|                 | 0.0810                                                       | 0.0810    | N.S            |                 |

(Table 10). shows variation in protein content in the juice across different ripening stages and varieties. The highest protein content was observed in the unripe stage of the Salimi cultivar at 1.716%, while the lowest was recorded in the Shukar cultivar at 1.610%. During the semi-ripe stage, Salimi again had the highest protein level (1.673%), while Shukar showed the lowest (1.520%). In the ripe stage, Salimi maintained the highest content (1.626%), and the lowest was recorded in Shukar at 1.571%. The results revealed significant differences among ripening stages.

**Table 10.** Protein percentage in pomegranate juice (%)

| Varieties       | Protein content across pomegranate fruit ripening stages (%) |           |                | Mean varieties  |
|-----------------|--------------------------------------------------------------|-----------|----------------|-----------------|
|                 | Unripe Stage (%)                                             | Semi-Ripe | Ripe Stage (%) |                 |
| Sulaimi         | 1.716                                                        | 1.673     | 1.626          | 1.672           |
| Khudrawi        | 1.693                                                        | 1.616     | 1.576          | 1.628           |
| Shukar          | 1.610                                                        | 1.520     | 1.510          | 1.546           |
| Mean ripe stage | 1.673                                                        | 1.603     | 1.571          |                 |
| L.S.D5%         | Ripe stage                                                   | Varieties | Interaction    | Correlation = - |
|                 | 0.022                                                        | 0.022     | N.S            |                 |

Jalal et al. (2018) reported that the protein content in the powder of ripe pomegranate peels was 3.26%. In a study conducted by Kareem and Naji (2022) to assess the chemical

The unripe stage recorded the highest average protein content at 2.557%, while the ripe stage showed the lowest average content at 2.300%. Additionally, significant differences were observed among varieties. The Salimi cultivar recorded the highest mean protein content at 2.539%, whereas the Shukar cultivar had the lowest at 2.309%. A highly significant negative correlation was found between infestation percentage and protein content in the pulp across ripening stages, suggesting that higher protein levels may enhance resistance to *Ectomyelois ceratoniae*.

The highest average protein content was observed in the unripe stage (1.673%), while the lowest was found in the ripe stage (1.571%). Likewise, significant differences were found among varieties. Salimi cultivar consistently showed higher mean protein levels (1.672%) than variety. Shukar (1.546%) A strong negative correlation ( $p < 0.01$ ) was observed between juice protein content and fruit infestation percentage throughout maturation., suggesting that increased protein levels may contribute to reduced susceptibility to *Ectomyelois ceratoniae*.

composition of the peels of various plants, pomegranate peel was found to contain 4.37% protein.

**Carbohydrate percentage in pomegranate fruits:** The result showed variations in carbohydrate content across ripening stages and varieties (Table 11). In the unripe stage, the highest carbohydrate content was recorded in the peel of Shukar cultivar (68.428%), while the lowest (67.923%) was in Salimi. In the semi-ripe stage, Shukar again had the highest (68.893%) and Salimi the lowest (68.323%) carbohydrate concentration. The lowest concentration was seen in Salimi (69.097), while the highest was in Shukar (69.010) at ripe stage. The results showed a significant difference among the ripening stages. The carbohydrate content (69.248%) at the ripe stage was higher than that (68.428%)

at the unripe stage. Also, cultivar differences were significant. Shukar cultivar (69.014%) indicated a maximum and Salimi cultivar (68.448%) showed a minimum mean content of carbohydrate compared to other varieties. The percentage of infestation had a positive correlation with carbohydrate content in the peel during the ripening stages, meaning higher carbohydrates percentage may be susceptible to the attack of *Ectomyelois ceratoniae*, infestation rate of pomegranate varieties was higher at the maturity of fruit, apparently because of the lower totals polyphenol, flavonoid contents, titratable acidity, and the higher total sugar and TSS content (Guess et al., 2003).

**Table 11.** Carbohydrate percentage in pomegranate fruit peel (%)

| Varieties       | Carbohydrate percentage across pomegranate fruit ripening stages (%) |           |                | Mean varieties |
|-----------------|----------------------------------------------------------------------|-----------|----------------|----------------|
|                 | Unripe Stage (%)                                                     | Semi-Ripe | Ripe Stage (%) |                |
| Sulaimi         | 67.923                                                               | 68.323    | 69.097         | 68.448         |
| Khudrawi        | 68.610                                                               | 68.777    | 69.247         | 68.878         |
| Shukar          | 68.750                                                               | 68.893    | 69.400         | 69.014         |
| Mean ripe stage | 68.428                                                               | 68.664    | 69.248         |                |
| L.S.D5%         | Ripe stage                                                           | Varieties | Interaction    | Correlation =  |
|                 | 0.0602                                                               | 0.0602    | 0.1042         |                |

(Table 12). shows variation in carbohydrate during ripening stages and varieties. In the unripe stage, the highest carbohydrate content in the pulp was recorded in the Shukar cultivar at 68.323%, while the lowest was in Salimi at 65.187%. During the semi-ripe stage, Shukar again showed the highest carbohydrate level (68.777%), and Salimi the lowest (65.243%). In the ripe stage, Shukar maintained the highest value of 68,893%, while Salimi recorded the lowest 68,323%. The results revealed significant differences between maturation stages, with the lowest average carbohydrate content seen in the ripe stage

(68,664%) and the lowest in the lowest unripe (65,717%). Similarly, significant differences were found among varieties. The average carbohydrate was observed greatest in Shukar cultivar (67.171%) and lowest in Salimi cultivar (66.251%). There was a significant positive correlation regarding infestation percentage with carbohydrate content in the pulp at the stages of ripening, which suggests that high carbohydrate percentage may have a direct relationships which high susceptibility to infestation from *Ectomyelois ceratoniae* (Rat and Mamay, 2024).

**Table 12.** Carbohydrate percentage in pomegranate pulp (%)

| Varieties       | Carbohydrate percentage across pomegranate fruit ripening stages (%) |           |                | Mean varieties |
|-----------------|----------------------------------------------------------------------|-----------|----------------|----------------|
|                 | Unripe Stage (%)                                                     | Semi-Ripe | Ripe Stage (%) |                |
| Sulaimi         | 65.187                                                               | 65.243    | 68.323         | 66.251         |
| Khudrawi        | 65.837                                                               | 66.120    | 66.493         | 66.911         |
| Shukar          | 68.323                                                               | 68.777    | 68.893         | 67.171         |
| Mean ripe stage | 65.717                                                               | 65.952    | 68.664         |                |
| L.S.D5%         | Ripe stage                                                           | Varieties | Interaction    | Correlation =  |
|                 | 0.1419                                                               | 0.1419    | 0.2458         |                |

Results in (Table 13). show variation in carbohydrate content in the juice across fruit ripening stages and varieties. In the unripe stage, the highest carbohydrate content was recorded in the Shukar cultivar at 18.703%, while the lowest was in the Salimi cultivar at 16.967%. During the semi-ripe stage, Shukar again exhibited the highest value (18.713%), and Salimi the lowest (18.273%). In the ripe stage, variety. Shukar showed the highest carbohydrate concentration (18.940%), while variety. Salimi had the lowest (18.350%). The results revealed a significant difference

between ripening stages, with carbohydrate content increased from unripe (18.119%) to ripe stages (18.677%). Furthermore, Varieties - variety. Shukar consistently showed higher mean carbohydrate levels (18.786%) than variety. Salimi (17.863%). A high significant positive correlation ( $p < 0.01$ ) was observed between juice carbohydrate content and infestation percentage throughout fruit development, the reason is the high percentage of carbohydrates as the pomegranate ripens (Zarei et al., 2011).

**Table 13.** Carbohydrate percentage in pomegranate juice (%)

| Varieties       | Carbohydrate percentage across pomegranate fruit ripening stages (%) |           |                | Mean varieties |
|-----------------|----------------------------------------------------------------------|-----------|----------------|----------------|
|                 | Unripe Stage (%)                                                     | Semi-Ripe | Ripe Stage (%) |                |
| Sulaimi         | 16.967                                                               | 18.273    | 18.350         | 17.863         |
| Khudrawi        | 18.687                                                               | 18.507    | 18.740         | 18.644         |
| Shukar          | 18.703                                                               | 18.713    | 18.940         | 18.786         |
| Mean ripe stage | 18.119                                                               | 18.498    | 18.677         |                |
| L.S.D5%         | Ripe stage                                                           | Varieties | Interaction    | Correlation =  |
|                 | 0.1340                                                               | 0.1340    | 0.2322         |                |

Guess et al. (2003) Pomegranate juice carbohydrate content ranges from 25% to 65%. In the other hand with Amoros et al. (2000) reported that pomegranate juice contain a total carbohydrate in a range from 16% to 20%.

## CONCLUSION

The current study revealed that *Ectomyelois ceratoniae* infestation is highly dependent on stage of ripening and chemical characteristics in pomegranate fruits, being responsible for different levels of infestation among pomegranate varieties. The Salimi cultivar maintained the lowest infestation levels and greatest concentrations of total phenols, tannins and proteins, had both highest total phenols, tannins, and proteins in peel, pulp and juice. The Shukar cultivar on the other hand revealed higher infestation percentage and low amounts of these compounds. A highly significant negative correlation of infestation percentage with phenolic, tannin and protein content, while carbohydrate level had a strong positive correlation with infestation. The results underscore the significant role of biochemical composition of the different varieties that contributed to cultivar resistance and demonstrate that

selection for genotypes with more phenolics and tannins could represent a useful strategy for the management of *E. ceratoniae* in pomegranate orchards.

## ACKNOWLEDGEMENT

The authors sincerely acknowledge the College of Agriculture, University of Kerbala, Iraq, and the College of Agricultural Engineering Sciences, University of Baghdad, Iraq, for providing the facilities and technical support necessary to complete this research. The authors also express their appreciation to all staff members who assisted in field sampling, laboratory analyses, and data collection.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHOR/S DECLARATION

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have been obtained and are available upon request.

All authors have read and approved the final version of the manuscript and agree to its submission for publication.

The corresponding author has signed the Ethical Approval Statement on behalf of all authors.

### **ETHICAL CLEARANCE AND ANIMAL WELFARE**

This study did not involve human participants or vertebrate animals requiring ethical approval. The research was conducted on naturally occurring insect populations and pomegranate fruits under field and laboratory conditions. All experimental procedures were carried out in accordance with institutional research guidelines and accepted standards for entomological research.

### **FUNDS**

This research received no external funding.

### **AUTHOR'S CONTRIBUTION STATEMENT**

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Field sampling and laboratory experiments: Manar A. Abbas

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## تأثير الخصائص الكيميائية لأصناف الرمان على الإصابة بدودة ثمار الرمان

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### المستخلص

أجريت الدراسة للتعرف على قابلية ثلاثة أصناف محلية من الرمان (سليمي، خضراوي، وشكر) للإصابة بحشرة دودة ثمار الرمان *Ectomyelois ceratoniae* أثناء مراحل نضج الثمار المختلفة وتحديد بعض الخصائص الكيميائية لهذه الأصناف ودورها بإصابة الحشرة. بينت النتائج تباين معدلات الإصابة بالرمان بين الأصناف المدروسة كان الصنف شكر أكثر الأصناف إصابة بلغت نسبة الإصابة 50.18% بينما الصنف سليمي هو الأقل إصابة إذ بلغ 35.47% وبين مراحل نضج الثمار حيث كانت المرحلة الناضجة هي الأكثر إصابة بلغت 72.11% بينما المرحلة الغير ناضجة هي الأقل إصابة بلغت 25.44%. أظهرت النتائج اختلاف في الصفات الكيميائية أثناء نضج ثمار الرمان إذ بينت النتائج وجود معامل ارتباط موجب بين نسبة الإصابة وكمية الكربوهيدرات، ووجود علاقة ارتباط سالبة بين نسبة الإصابة والفينولات الكلية والتانين والبروتين مع تقدم نضج الثمار.

**الكلمات المفتاحية:** البروتين، التانين، الفينولات، الكربوهيدرات، مراحل النضج