



## Influence of Pomegranate Butterfly, *Virachola livia* (Klug) (Lepidoptera: Lycaenidae) Infestation on Chemical Composition of Pomegranate Fruits

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### Abstract

The pomegranate butterfly, *Virachola livia* (Klug), is one of the most important pests in Egypt affects the quantity and quality of fruits. At the end of the season, 20 healthy fruits and 20 infested fruits were collected from Manfaloty, Higazy and Araby cultivars to determine the effect of infestation on some chemical compositions of pomegranate fruits (juice and peels). Results showed that the infestation of pomegranate butterfly reduced the percentage of total soluble solids (14.73, 13.93 and 13.90%), TSS/acidity ratio (12.42, 11.71 and 8.49%), total sugars (12.92, 12.48 and 12.34%) and total reducing sugars (11.32, 11.03 and 10.33%) and caused a significant increase of total titratable acidity (1.19, 1.39 and 1.54) in the infested fruits compared to the healthy fruits TSS (16.43, 16.33 and 14.37%), TSS/acidity ratio (14.41, 14.24 and 10.97%), TS (14.88, 14.62 and 13.42%) and TRS (14.13, 13.23 and 11.19%) and TTA (1.13, 1.26 and 1.35) of Mnafaloty, Higazy and Araby cultivars, respectively. In addition, the infestation of this pest also affects the chemical composition of pomegranate peels which reduced the percentage of moisture (72.96, 65.53 and 61.78%), protein (4.52, 4.07 and 3.62%), Potassium (12.70, 10.72 and 10.01 g/kg) and caused a significant increase in total carbohydrates (28.89, 27.27 and 12.93%), Calcium (3.26, 3.70 and 3.95 g/kg) and pH values (3.87, 3.90 and 4.19) in the infested fruits compared to the healthy fruits moisture (74.53, 67.08 and 62.76%), protein (4.93, 4.28 and 4.91%), potassium (15.92, 13.16 and 12.21g/kg), total carbohydrates (22.37, 25.51 and 12.21%), Calcium (2.59, 2.54 and 3.21g/kg) and pH values (4.09, 3.99 and 4.46). These results show that the pomegranate butterfly infestation affects the chemical composition of fruits and decreased the economic importance of pomegranate. Therefore, an integrated control program must be developed for this pest to improve the quality and quantity of pomegranate fruits and to reduce its damage to the crop.

**Keywords:** Pomegranate, *Virachola livia*, chemical composition, cultivars

## Introduction

Pomegranate, *Punica granatum* L. is an important fruit crop of arid and semiarid regions of the world. In Egypt, pomegranate is one of the most important crops, making up the primary income of the farmers, especially in Upper Egypt. Pomegranate is also used by International Medical Institutions, implying the significance it represents in medicinal uses, and that such Institutions are convinced by the medical benefits of pomegranate (Holland *et al.* 2009; Marzooq & El-Iraqi, 2015). The total cultivated area, production and productivity of pomegranate in Egypt were 80098 feddan (76% of these areas located in Assiut Governorate), 649900 ton (91% produced in Assiut Governorate), and 8.44 ton/feddan, respectively in 2019 year (Ahmed, 2021). There is a growing interest in increasing its planting area and introducing high juice yielding new cultivars such as Wonderful to replace the old ones. Pomegranate is the 5<sup>th</sup> most exported crop in Egypt during 2020 (482866 Tons) based on the report of the Central Administration for Agricultural Quarantine (Ahmed, 2021).

Several pests attack pomegranate trees such as sucking pests like whiteflies, aphids, mealybugs, scale insects, thrips, mites, and pomegranate butterfly which reduced pomegranate fruit quality and yield (Awadallah *et al.*, 1971; Mote *et al.*, 1992; Sayed & Temarek, 2007; Holland *et al.*, 2009; Abd-Ella, 2015; Elango *et al.*, 2021). The quality of pomegranate export fruits depends on the size, color, free from pest infestation and pesticide residue levels. To improve the quality of pomegranate fruits and to increase the exportation, it is necessary to use biopesticides and safe alternative practices for controlling pomegranate butterfly and sucking pests.

The pomegranate butterfly, *Virachola livia* (Klug) (Lepidoptera: Lycaenidae) is one of the most important pests in Egypt which affecting the quantity and quality of fruits (Abd-Ella, 2015). It caused serious damage in many countries of the world, such as Jordan (Obeidat and Akkawi, 2002), Oman (Abbas *et al.*, 2008; Kinawy *et al.*, 2008), Egypt (Sayed *et al.*, 2010; Abd-Ella, 2015), Tunisia (Ksentini *et al.*, 2011) and Algeria (Beddief *et al.*, 2020). This species is also declared as a pest of the date palm in Morocco (Sedra, 2003); Egypt (Sayed and Temarek, 2007), and Tunisia, where it was detected for the first time in 2011 (Zouba & Chermiti, 2015).

Infestation of pomegranate butterfly is one of the factors that affect fruit health. Oviposition of insect, puncture, larva feeding can affect fruit drop (Umeh *et al.*, 2004), hurried maturity (Jayanthi *et al.*, 2015), changes in peel color of fruit (Jayanthi *et al.*, 2015), changes in the chemical composition of juices (Omoloye *et al.*, 2016), growth pathogen in fruit peel (Engelbrecht *et al.*, 2004; Omoloye *et al.*, 2016) and damage to fruit pulp (Jayanthi *et al.*, 2015; Omoloye *et al.*, 2016). The damage caused by pomegranate butterfly, *V. livia* infestation may affect the quality of fruit for commercial sale and consumption. The aim of the present study is to determine the Effect of pomegranate butterfly infestation on the chemical composition of pomegranate fruits (juice and peels). Chemical compositions (total soluble solids (TSS), total titratable acidity, TSS / acidity, total sugars (TS) and

total reducing sugars (TRS) (%)) of juices, which were extracted from healthy and infested fruits of Manfaloty, Higazy and Araby cultivars were determined. Chemical compositions (moisture, protein, total carbohydrates%, K, Ca and pH) of healthy and infested pomegranate fruit peels of Manfaloty, Higazy and Araby cultivars were also assessed. This data could evaluate the quantity and quality loss of pomegranate fruit for consumption and processing.

## **Materials and Methods**

### **Experimental field sites**

The experiment was carried out at the Experimental Orchard, Pomology Department, Faculty of Agriculture, Assiut University on Manfaloty, Higazy and Araby pomegranate cultivars. The soil was heavy loam and regular agricultural managements were applied to all experimental trees as recommended. The trees were 5m apart from each other and they were 34 year-old at the commencement of the experiment. The trees of the orchards were of uniform vigor and size.

### **Effect of *V. livia* infestation on some chemical composition of pomegranate fruits**

At the end of the season, 20 healthy fruits and 20 infested fruits by *V. livia* were collected from each cultivar to determine the effect of infestation on some chemical compositions of pomegranate fruits (juice and peels).

### **Chemical characterization of pomegranate juices**

Chemical characterizations (total soluble solids (TSS), total titratable acidity, TSS / acidity, total sugars (TS) and total reducing sugars (TRS) (%)) of juices isolated from healthy and infested fruits by pomegranate butterfly, *V. livia* attack pomegranate fruit Manfaloty, Higazy and Araby cultivars were determined in Pomology Department, Faculty of Agriculture, Assiut University. Total titratable acidity (TTA) was determined by titration with 0.1N NaOH according to the method described by (AOAC, 1990). The results were expressed as ml equivalents NaOH/100gm D.W. Total soluble solids (TSS as%) was quantified using the hand refractometer and then, TSS/Acid ratio was calculated. Percentages of total and reducing sugars in the juice were estimated according to AOAC (1990).

### **Chemical characterization of pomegranate peels**

Chemical characterization (moisture, protein, total carbohydrates %, K, Ca and pH) of pomegranate peels healthy and infested fruits by pomegranate butterfly, *V. livia* attack pomegranate fruit cultivars Manfaloty, Higazy and Araby were determined in Central Laboratory for Chemical Analysis, Faculty of Agriculture, Assiut University. Moisture, crude protein and total carbohydrates were determined as described in the (AOAC, 2000). Calcium was determined by Inductively Coupled Plasma Emission Spectrometer (iCP6200). Potassium was determined using the Flam Photometer. Nitrogen was determined using the Kjeldahl Unit. Total Carbohydrates were determined using the Spectrophotometer.

### Statistical analysis

One-way ANOVA was used to analyze the data and presented as mean  $\pm$  SEM. Statistical analysis and Figures were done using Graph Pad Prism 5<sup>TM</sup> software (San Diego, CA). Duncan's multiple range test (DMRT) ( $P < 0.05$ ) was separated of means, whenever differences were indicated.

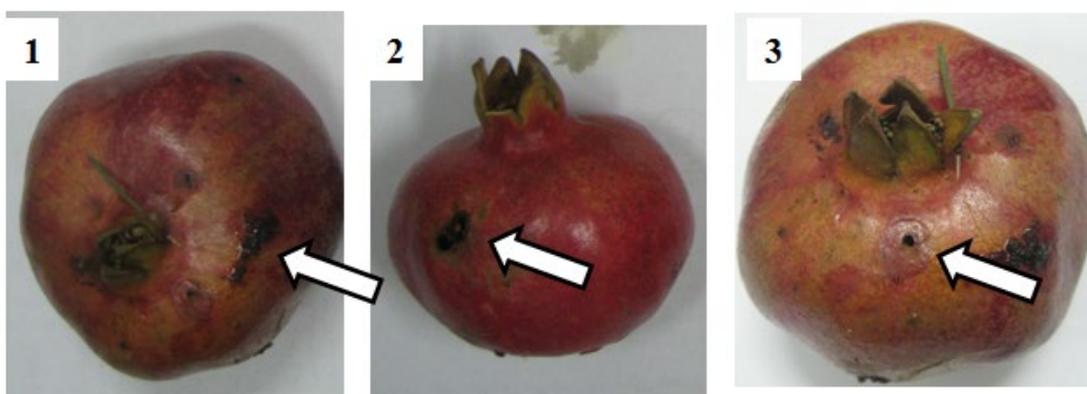
### Results and Discussion

In this work, the pomegranate butterfly, *V. livia* is common pest in Assiut pomegranate orchards that primarily cause damage to fruits. *V. livia* larvae damage are described as a red circular shape of 1-2 cm diameter which turns into dark color at the late-developing stage (Fig. 1B) compared to the healthy pomegranate fruits (Fig. 1A). In addition, the skin becomes a black color of the fruits because larvae produce wastes where many fungi grow on fruits.

#### (A) Healthy pomegranate fruit cultivars



#### (B) Infested fruits by pomegranate butterfly, *V. livia*



**Fig. 1.** Healthy and infested fruits by pomegranate butterfly, *V. livia* attack pomegranate fruit cultivars Manfaloty (1), Higazy (2) and Araby (3) in Assiut.

To our knowledge, there is a lack of information about the influence of the pomegranate butterfly, *V. livia* effect on the chemical composition of pomegranate fruits. Thus, the aim of this work is to evaluate for the first time the effect of pomegranate butterfly on the chemical composition of pomegranate fruits (juice

and peels) from healthy and infested fruits by pomegranate butterfly, *V. livia* attack pomegranate fruit Manfaloty, Higazy and Araby cultivars.

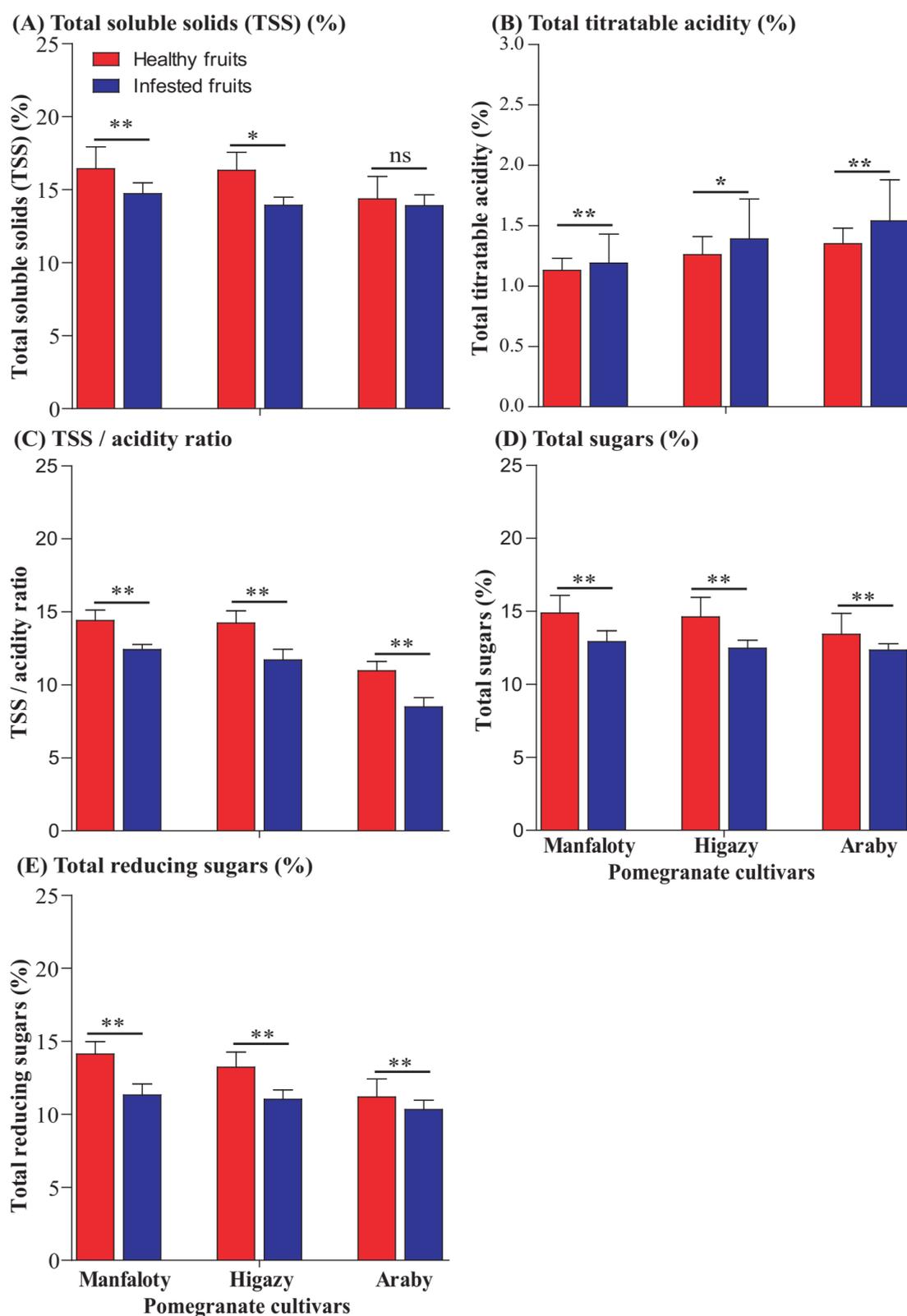
### **Effect of pomegranate butterfly, *V. livia* infestation on pomegranate juice quality**

#### **Effect of pomegranate butterfly, *V. livia* on total soluble solids (TSS)**

TSS in pomegranate fruit cultivars were altered due to *V. livia* infestation (Fig. 2A). Total soluble solids decreased by 10.35, 14.7 and 3.27% in pomegranate-infested fruit cultivars, Manfaloty, Higazy and Araby respectively. Results showed that the infestation of pomegranate butterfly caused a significant reduction in the percentage of total soluble solids (14.73, 13.93 and 13.90%), compared to the healthy fruits (16.43, 16.33 and 14.37%), of Mnafaloty, Higazy and Araby cultivars respectively. *V. livia* infestation changed the chemical composition of pomegranate fruit for each cultivar. However, each cultivar presented different chemical changes and with different intensities between them. The feeding of pomegranate butterfly larvae caused significant chemical changes such as total soluble solids. Omoloye *et al.* (2016) reported that the oranges infested by fruit flies in the field caused significant damage to the metabolic structure and enzymatic of the fruit, which was also observed from larval feeding.

#### **Effect of pomegranate butterfly, *V. livia* on total titratable acidity (TTA) and TSS/ acidity ratio**

Chemical analyses of total titratable acidity (TTA) on pomegranate fruit healthy and infested by pomegranate butterfly were shown in Figure (2B). Obtained results show that there was a high significant increase of the total titratable acidity (TTA) percentage in infested fruits (1.54 and 1.39%) on Araby and Higazy cultivars, whereas there was a significant increase (1.19%) on Manfaloty cultivar compared to healthy fruits (1.35, 1.26 and 1.13%) on Araby, Higazy and Manfaloty cultivars, respectively. A significant decrease of TTA percentage (about 5.04, 9.35 and 12.33%) on Manfaloty, Higazy and Araby cultivars respectively. Omoloye *et al.* (2016) stated that the changes in acidity observed mainly in pomegranate fruit cultivars may also be caused by larval feeding and extracellular digestive activities of bacteria that degrade components of nutritional fruit. The infestation of pomegranate butterfly caused a significant decrease in TSS/acidity ratio (12.42, 11.71 and 8.49%) in pomegranate infested fruit cultivars, Manfaloty, Higazy and Araby respectively compared to the healthy fruits (14.41, 14.24 and 10.97%) of Mnafaloty, Higazy and Araby cultivars, respectively (Fig. 2C). Pomegranate fruit quality is reduced by the feeding larvae of the *V. livia* for fresh consumption. Jayanthi *et al.* (2015) showed that the losses for fresh consumption are associated with firmness reduction and accelerated maturation. Consequently, the changes in fruit flavor result and reduction of shelf life, due to the infestation affects the ratio TSS/ TA (Omoloye *et al.*, 2016).



**Fig. 2.** Chemical characterization of TSS % (A), TTA % (B), TSS / acidity ratio (C), TS (D) and TRS % (E) of juices isolated from healthy and infested fruits by pomegranate butterfly, *V. livia* attack pomegranate fruit Manfaloty, Higazy and Araby cultivars at Assiut governorate. Data are expressed as means  $\pm$  stander error (SE), ns: non-significant  $p > 0.05$ , \* $p < 0.05$ , \*\* $p < 0.01$ .

### **Effect of pomegranate butterfly, *V. livia* on total sugars (TS) and total reducing sugars (TRS)**

Analyses of total sugars (TS) and total reducing sugars (TRS) on pomegranate fruit healthy and infested by pomegranate butterfly were shown in Figure (2 D and E). Total and total reducing sugars were altered by *V. livia* infestation, which caused a significant reduction (12.92, 12.48 and 12.34%) and (11.32, 11.03 and 10.33%) in the infested fruits compared to the healthy fruits TS (14.88, 14.62 and 13.42%) and TRS (14.13, 13.23 and 11.19%) of Manfaloty, Higazy and Araby cultivars, respectively.

### **Effect of pomegranate butterfly, *V. livia* infestation on pomegranate peels chemical compositions**

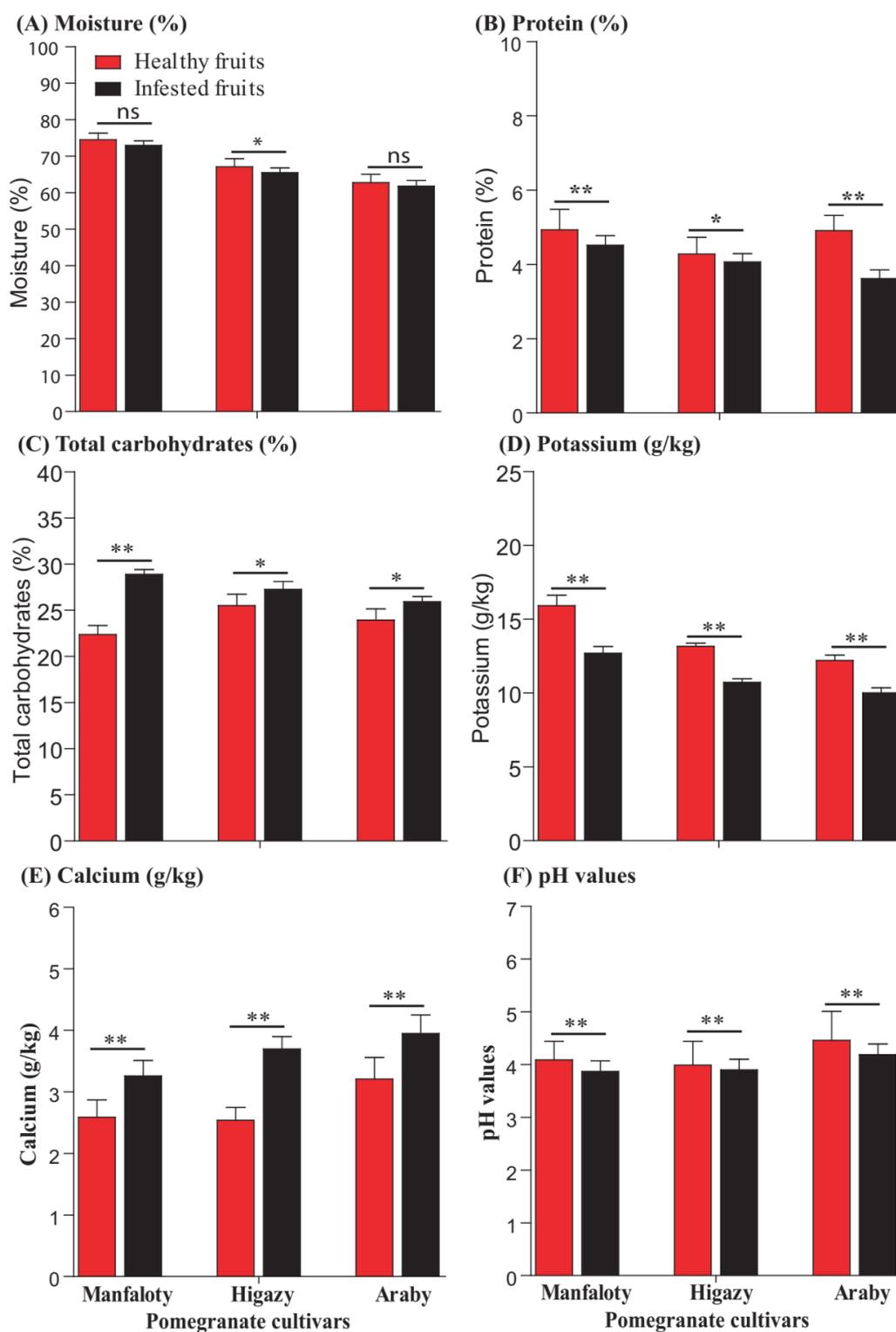
Peel chemical compositions of pomegranate fruit cultivars were altered due to *V. livia* infestation (Fig. 3 A-F). Moisture (%), protein (%), total carbohydrates (%), potassium (g/kg), calcium (g/kg) and pH values of peels pomegranate healthy and infested fruits by pomegranate butterfly, *V. livia* attack pomegranate fruit cultivars Manfaloty, Higazy and Araby were determined.

### **Effect of pomegranate butterfly, *V. livia* on Moisture percentage**

Figure (3 A) moisture percentage composition of pomegranate fruit cultivars Manfaloty, Higazy and Araby obtained from healthy and infested fruits by *V. livia* are presented. No significant differences in moisture percentage composition between the healthy (74.53 and 62.76%) and infested (72.96 and 61.78%) fruits on Manfaloty, and Araby cultivars, whereas a significant difference between the healthy (67.08%) and infested (65.53%) on Higazy cultivar. A non-significant decrease in moisture percentage (about 1.37, and 1.56 %) on Manfaloty, and Araby cultivars, whereas a significant decrease (about 2.31%) on Higazy cultivar.

### **Effect of pomegranate butterfly, *V. livia* on protein percentage**

Data in Figure (3 B) summarize the total protein percentage was detected by chemical analysis in healthy and infested fruits by *V. livia* of three pomegranate cultivars, Manfaloty, Higazy and Araby. Moreover, the results show that there was a significant decrease in the total protein percentage in infested fruits (4.52, 4.07 and 3.62 %) compared with healthy fruits (4.93, 4.28 and 4.91 %) on Manfaloty, Higazy and Araby cultivars respectively. A high significant decrease in the total protein percentage (about 26.27 and 8.32 %) on Araby and Manfaloty, whereas a significant decrease in Higazy cultivars was about 4.91%. Results indicated that the pomegranate butterfly attack did influence the total protein percentage in peel fruits of all pomegranate cultivars.



**Fig. 3.** Chemical characterization of moisture % (A), protein % (B), total carbohydrates % (C), Potassium (g/kg) (D), Calcium (g/kg) (E), and pH values (F) of peels pomegranate healthy and infested fruits by pomegranate butterfly, *V. livia* attack pomegranate fruit cultivars Manfaloty, Higazy and Araby at Assiut governorate. Data are expressed as means  $\pm$  stander error (SE), ns: non-significant  $p > 0.05$ , \* $p < 0.05$ , \*\* $p < 0.01$ .

### **Effect of pomegranate butterfly, *V. livia* on total carbohydrate percentage**

*V. livia* damage caused changes in the total carbohydrates (%) of three pomegranate cultivars, Manfaloty, Higazy and Araby compared to the healthy fruits (Fig. 3C). Moreover, the results show that there was a highly significant increase in the total carbohydrates (%) in infested fruits (28.89%) on Manfaloty cultivar compared with healthy fruits (22.37%). Whereas, a significant decrease in total carbohydrates percentage (27.27 and 25.93%) compared the healthy fruits (25.51 and 23.93%) in Higazy and Araby cultivars, respectively. A high significant increase in total carbohydrates percentage (about 22.56%) on Manfaloty cultivar and a significant increase (about 6.45 and 7.71%) on Higazy and Araby cultivars, respectively. Results indicated that the pomegranate butterfly attack influenced the total carbohydrates percentage in peels pomegranate of Manfaloty, Higazy and Araby compared to the healthy fruits.

### **Effect of pomegranate butterfly, *V. livia* on potassium composition**

The mineral contribution of pomegranate peels is characterized by the presence of potassium and calcium. The pomegranate butterfly, *V. livia* infestation caused a high decrease in potassium composition (12.70, 10.72 and 10.01 g/kg) compared to the healthy fruits (15.92, 13.16 and 12.21 g/kg) in Manfaloty, Higazy and Araby cultivars, respectively (Fig. 3D). A high significant decrease in potassium composition (about 20.23, 18.54 and 18.02 %) on Manfaloty, Higazy and Araby cultivars, respectively.

### **Effect of pomegranate butterfly, *V. livia* on Calcium composition**

The calcium composition of pomegranate peels of pomegranate cultivars was altered due to *V. livia* infestation (Fig. 3 E). This infestation caused a high raised in calcium composition (3.26, 3.70 and 3.95 g/kg) compared to the healthy fruits (2.59, 2.54 and 3.21 g/kg) in Manfaloty, Higazy and Araby cultivars, respectively. A high significant increase in calcium composition (about 20.55, 31.35 and 18.73 %) on Manfaloty, Higazy and Araby cultivars, respectively.

### **Effect of pomegranate butterfly, *V. livia* on pH values**

*V. livia* infestation caused changes in pH in peels of pomegranate fruit cultivars (Fig. 3F), decreasing the values by 3.87, 3.90 and 4.19 compared to the healthy fruits at 4.09, 3.99 and 4.46 in Manfaloty, Higazy and Araby cultivars, respectively. A high significant increase in pH values (about 5.38, 2.26 and 6.05 %) on Manfaloty, Higazy and Araby cultivars, respectively.

### **Conclusion**

In conclusion, data achieved in this study showed that the infestation of pomegranate butterfly caused a significant reduction in the percentage of TSS, TSS/acidity ratio, TS and TRS and caused a significant increase of TTA in the infested fruits compared to the healthy fruits of Mnaloty, Higazy and Araby pomegranate cultivars respectively. In addition, the infestation of this pest also affects the chemical composition of pomegranate peels which caused a significant reduction in the percentage of moisture, protein, potassium composition and

caused a significant increase in total carbohydrates percentage, calcium composition and pH values in the infested fruits compared to the healthy fruits. However, results showed that the pomegranate butterfly infestation affects the chemical composition of fruits and decreased the economic importance of pomegranate. Therefore, an integrated control program must be developed for this pest to improve the quality and quantity of pomegranate fruits and reduce its damage to the crop. This data can help evaluate of fresh fruit quality for consumption and processing. Hence, the relationship between pomegranate butterfly, *V. livia*/pomegranate cultivar fruits might influence chemical characteristic changes in fresh fruits of pomegranate.

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## تأثير الإصابة بحشرة ابي دقيق الرمان على المكونات الكيميائية لثمار الرمان

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### الملخص

تعتبر حشرة ابي دقيق الرمان من اخطر الآفات التي تصيب الرمان في مصر حيث تؤثر على كمية ونوعية الثمار. في نهاية الموسم جمعت 20 ثمرة خالية من الاصابة و 20 ثمرة مصابة من أصناف الرمان المنفلوطي والحجازي والعربي لتحديد تأثير الإصابة على المكونات الكيميائية لثمار الرمان في العصير والقشور. أظهرت النتائج أن الإصابة تسببت في انخفاض معنوي في نسبة المواد الصلبة الذائبة الكلية (14.73، 13.93، 13.90%) ومعدل المواد الصلبة الذائبة / الحموضة (12.42، 11.71، 8.49%) السكريات الكلية (12.92، 12.48، 12.34%) والسكريات المختزلة الكلية (11.32، 11.03، 10.33%) وتسببت في زيادة معنوية في الحموضة الكلية (1.19، 1.39، 1.54) في الثمار المصابة مقارنة بالثمار السليمة حيث كانت المواد الصلبة الذائبة الكلية (16.43، 16.33، 14.37%)، نسبة المواد الصلبة الذائبة / الحموضة (14.41، 14.24، 10.97%)، السكريات الكلية (14.88، 14.62، 13.42%) والسكريات المختزلة الكلية (14.13، 13.23، 11.19%) والحموضة الكلية (1.13، 1.26، 1.35) في عصير ثمار أصناف المنفلوطي والحجازي والعربي على التوالي. كذلك الإصابة بهذه الآفة تؤثر أيضًا على التركيب الكيميائي لقشور الرمان مما أدى إلى انخفاض معنوي في نسبة الرطوبة (72.96، 65.53، 61.78%) والبروتين (4.52، 4.07، 3.62%) والبوتاسيوم (12.70، 10.72، 10.01 جم/كجم) وتسببت في زيادة معنوية في نسبة الكربوهيدرات الكلية (28.89، 27.27، 12.93%) والكالسيوم (3.26، 3.70، 3.95 جم/كجم) وقيم الأس الهيدروجيني (3.87، 3.90، 4.19) في الثمار المصابة مقارنة مع الثمار السليمة حيث كانت نسبة الرطوبة (74.53، 67.08، 62.76%)، البروتين (4.93، 4.28، 4.91%)، البوتاسيوم (15.92، 13.16، 12.21 جم/كجم)، الكربوهيدرات الكلية (22.37، 25.51، 12.21%)، الكالسيوم (2.59، 2.54، 3.21 جم/كجم) وقيم الأس الهيدروجيني (4.09، 3.99، 4.46). وتشير هذه النتائج إلى أن الإصابة بحشرة ابو دقيق الرمان يؤثر على التركيب الكيميائي للثمار ويقلل من الأهمية الاقتصادية للرمان. لذلك يجب وضع برنامج مكافحة متكامل لهذه الآفة لتحسين نوعية وكمية ثمار الرمان وخفض ضررها على هذا المحصول.