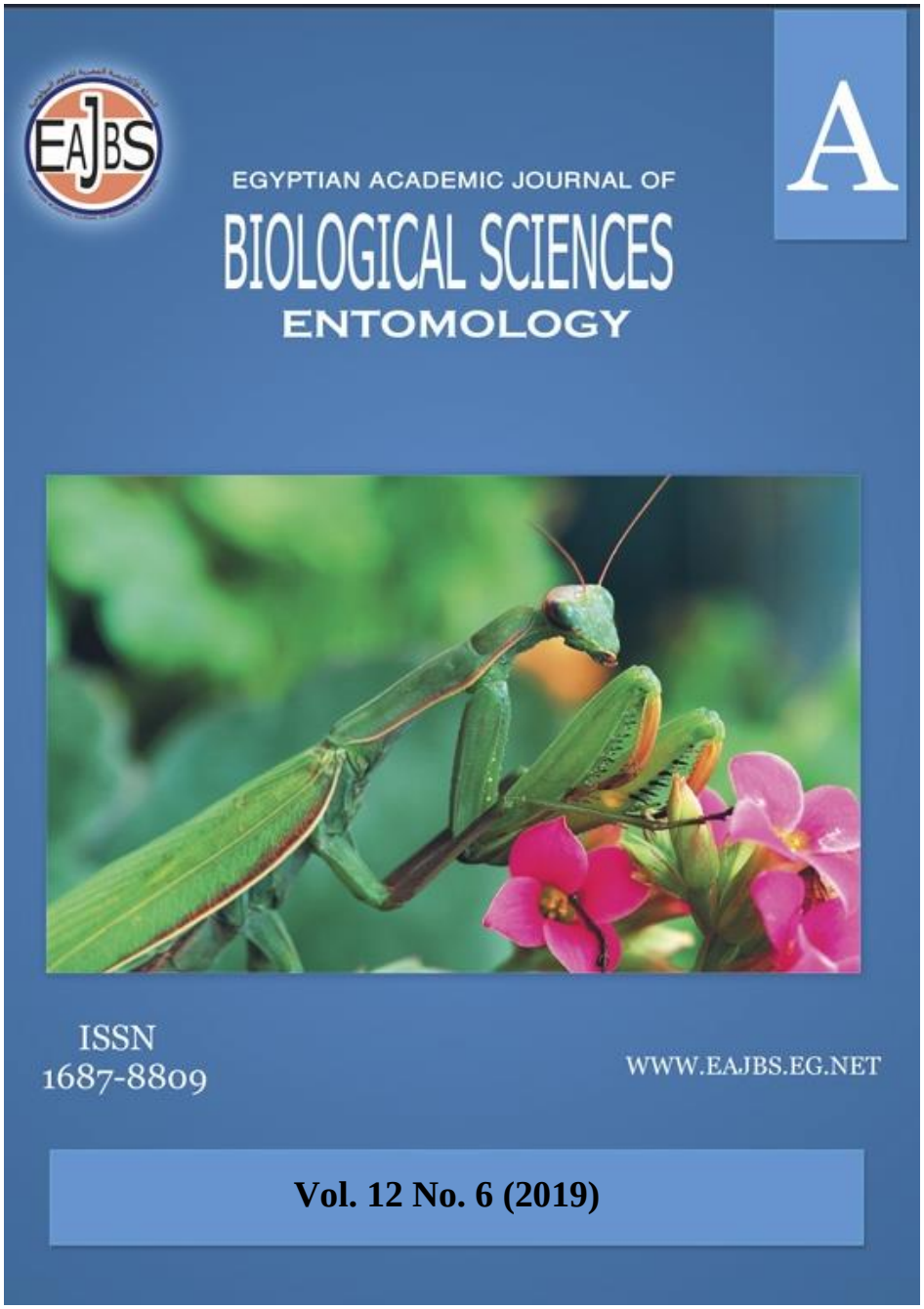

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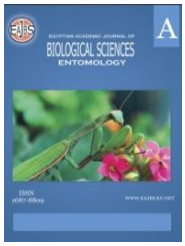

Effect of Infested Carnation Flowers by *Haplothrips cottei* and *Tetranychus urticae* on the Vase Life Period under Glasshouse Conditions

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ABSTRACT

This study was carried out to study effect of infested Carnation flowers (*Dianthus caryophyllus* L.) by Carnation thrips, *Haplothrips cottei* (Vuillet) (Thysanoptera: Thripidae) and *Tetranychus urticae* Koch (Acari: Tetranychidae) on the vase life period of Carnation flowers under glasshouse conditions at two locations (governorates), International Garden (Cairo Governorate) and El-Orman Garden, (Giza Governorate) during successive seasons 2018. This is because the vase life period is very important parameter in cut flowers. And there are many factors affected on the vase life period. Therefore this study divided into two parts, first part studied effect of infested Carnation flowers by *H. cottei* and *T. urticae* on the vase life period of Carnation flowers after picking. Second part studied effect of infested Carnation flowers by the same pests on the internal components of these flowers which correlated with vase life period such as total sugar and total protein. Results obtained showed that the infestation by *H. cottei* reduced the vase life period of carnation flowers after picking more the infestation by *T. urticae* compared to control (which non infested by the same pests). Also results showed that the infestation by *H. cottei* reduced total suger and total protein at the infested Carnation flowers more than the infestation by *T. urticae* compared to control. Lastly, results obtained showed that the infestation by *H. cottei* and *T. urticae* changed the number and arrange of the protein banding patterns (amino acids) of infested Carnation flowers petals compared to control.

INTRODUCTION

Carnation considers one of the most important cut flowers and ornamental plants in Egypt and all over the world which cultivated in the open field and under greenhouse conditions. Also, its cultivated area increased gradually during the last years, especially in the new reclaimed areas for purposes local consumption and exportation to the foreign markets. The human love to the dianthus due to their beautiful colors, style of flowers, smiles, and tolerant the inferable weather factors. Later dianthus flowers became one of the important components for international income for many countries all over the world through exporting these flowers to the different countries, Ali, A. *et al*. (2008)

Carnation plants infested with large scale of insects belong to many orders and families such as *Haplothrips cottei* and *Tetranychus urticae* which are considered important pests of carnation flowers and many other flowers. Jaskiewicz, B. (2010) who reported that the strong

infestation by *H.cottei* resulted in the deformation of stems, leaves, and flowers of Carnation plants. Derek, M. (2013) in Australia who reported that *H. cottei* and *T. urticae* are serious pests on Carnation flowers, and they feed mainly on the young leaves and developing flower-buds of Carnation flowers.

This study was carried out to study the effect of infested Carnation flowers *(Dianthus caryophyllus* L.) by *H. cottei* and *T. urticae* on the vase life period of Carnation flowers under glasshouse conditions at two locations, International Garden (Cairo Governorate) and El-Orman Garden, (Giza Governorate) during successive seasons 2018.

MATERIALS AND METHODS

Experimental Design:

This study was conducted on Carnation plants grown in two locations, International Garden (Cairo Governorate) and El-Orman Garden, (Giza Governorate) under glasshouse conditions during successive seasons 2018. The glasshouse in each garden with an area of 27x45 m of each one was divided into three parts, first part left as control, second part had by *Haplothrips cottei* and the third part had artificially infestation by *Tetranychus urticae*. Each part contains 5 plots (3x5 m^2) for each, and each part isolated completely from others. Carnation seedlings were planted in glasshouse conditions at the same time in November (the planting time of Carnation plants). All agricultural operations of irrigation and fertilization and others are completely identical in the two glasshouses were done without application of any insecticide.

Artificially infestation was done by *H. cottei* in the second part and by *T. urticae* in the third part with careful observation of the mean numbers of these pests during the plant growth period and especially during the flowering stage from February – August. At the end of the first growing season, 100 flowers were collected from each part at the two locations. At both of two glasshouses all post-harvest treatments were identical but conducted separately. Until the arrival of the flowers for the final stage, a stage put flowers in Wares glass (vase) where each group is divided into five containers respective 20 flowers per each one (vase) and in the presence of water only without adding any other materials prolong or reduce the period of the existence or the life of flowers in glassware. By taking into account the complete separation between the containers and control containers with daily monitoring of the status of flowers in both of the two glasshouses.

Effect of Insect Infestation by *H. cottei* and *T. urticae* on the Internal Components of Carnation Flowers:

These experiments were carried out to study effect of insect infestation by *H. cottei* and *T. urticae* on the vase life period of Carnation flowers through study effect of insect infestation by the same pests on the internal components of Carnation flowers specifically two elements (total sugars and total protein) which have strongly correlated with the vase life period.

Determination of Protein Banding Pattern:

Total protein Extraction:

Total proteins were extracted from 0.5 kg of fresh tissue of Carnation flowers. The tissues were ground in liquid nitrogen with a mortar and pestle. Then few mls of tris buffer extraction were added (1:2, tissue: buffer). The medium of extraction contained tris-HCL buffer (0.1mM tris, pH 7.5, 4mM B-mercaptoethanol, 0.1mM EDTA-Na_2, 10mM KCl and 10mM $MgCl_2$). The crude homogenate was centrifuged at 10.000xg for 20min. The supernatant was used for gel analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970).

Loading on a Gel:

Gel Preparation:

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 12.5% acrylamide and 0.8% bis-acrylamide running gel consisting of 0.375 M Tris-HCl (pH 8.8) and 0.1% SDS. Stacking gel (10 mm) was made using 4.5% acrylamide containing 0.8% bis-acrylamide in 0.125 M Tris–HCl (pH6.8) and 0.1% SDS. The electrophoresis buffer contained 0.025 M Tris-HCl, 0.19 glycine and 0.1% SDS. The samples were homogenized in 0.12M Tris-HCl (pH 6.8), 0.4 SDS, 10 B-mercaptoethanol, 0.02% bromophenol blue and 20% glycerol. The samples were then heated for 3min. in a boiling water bath before centrifugation. The gel was run under cooling at 90v for the first 15min, then 120v the next 0.5 hr and finally 150v for the remaining 1.5hr. Sheri, *et al*. (2000).

Sample Loading:

A known volume of protein sample was applied to each well by micropipette. Control wells were loaded with standard protein markers.

Electrophoresis Conditions:

The running buffer was poured into a pre-cooled (4°C) running tank. The running buffer was added in the upper tank just before running so that the gel was completely covered. The electrodes were connected to the power supply adjusted at 100 v until the bromophenol blue dye entered the resolving gel and then increased to 250v until the bromophenol blue dye reached the bottom of the resolving gel.

Gel Staining and Distaining:

After the completion of the run, the gel was placed in a staining solution consisting of 1g of Coomassie Brilliant Blue–R-250; 455 ml methanol; 90ml glacial acetic acid and completed to 1L with deionized distilled water. The gel was destained with 200ml destaining solution (100ml glacial acetic acid, 400ml methanol and completed to 1L by distilled water) and agitated gently on shaker. The destaining solution was changed several times until the gel background was clear.

Gel Analysis:

Gels were photographed using a Bio-Rad gel documentation system. Data analysis was obtained by Bio-Rad Quantity one Software version 4.0.3

Statistical Analysis:

In the experiments, the effect on the insect infestation by *H. cottei* and *T. urticae* on the vase life period of the Carnation flowers. And the effect of the infestation by the same pests on the total soluble sugar and total protein of the Carnation flowers were subjected to analysis of variance (ANOVA) and the means were compared by L.S.D. test at 0.05 level, using SAS program (SAS Institute, 1988). The sugar and protein were analyzed by High-Pressure Liquid Chromatograph (HPLC).

RESULTS AND DISCUSSION

This study was carried out to study effect of infested Carnation flowers (*Dianthus caryophyllus* L.) by Carnation thrips, *Haplothrips cottei* and *Tetranychus urticae* on the vase life period of Carnation flowers under glasshouse conditions at two locations (governorates), International Garden (Cairo Governorate) and El-Orman Garden, (Giza Governorate) during successive seasons 2018. This is because the vase life period is a very important parameter in cut flowers. And there are many factors affected by the vase life period. Therefore, this study divided into two parts, first part studied effect of infested Carnation flowers by *H. cottei* and *T. urticae* on the vase life period after picking. The second part studied effect of infested Carnation flowers by the same pests on the internal components of these flowers, which correlated with vase life periods such as total sugar and total protein.

Effect of the Insect Infestation by *H. cottei* and *T. urticae* on the Vase Life Period of Carnation Flowers:

This experiment was carried out to study the effect of infested Carnation flowers by *H. cottei* and *T. urticae* on the vase life period. Means of vase life period (flowers life after picking) for infested flowers by the two pests compared to control (non-infested) at the two examined locations were recorded.

Data tabulated in Table (1) show means of lifetime (vase life period) of the Carnation flowers which infested by *H. cottei* and *T. urticae* compared to control (non-infested) for the five varieties (colors) of Carnation flowers (yellow, red, pink, blue and white) at the two examined locations. Data obtained showed the means of vase life period of Carnation flowers in control which did not infest by any pests ranged from 9.8 to 11.6 days for the five varieties (colors) of Carnation flowers, means of vase life period of Carnation flowers which infested by *H. cottei* ranged from 4.8 to 6.5days, while means of vase life period of Carnation flowers which infested by *T. urticae* ranged from 6.9 to 8.2 days

Table (1): Effect of insect infestation by *H. cottei* and *T. urticae* on the vase life period of Carnation flowers after picking compared to control

Carnation	Vase life period / days				
	H. cottei	*T. urticae*	Control	F(0.05)	LSD
Yellow	6.2^{c}	8.2^{b}	11.6^{a}	345.43	1.032
Red	6.5^{c}	7.5^{b}	10.4^{a}	276.87	1.053
Pink	5.4^{c}	6.5^{c}	9.8^{a}	264.21	1.034
Blue	4.8^{c}	6.9^{c}	10.7^{a}	341.93	1.041
White	5.7^{c}	7.8^{b}	10.3^{a}	269.17	1.067

Means within columns bearing different subscripts are significantly different ($P< 0.05$)

Statically analysis shows highly significant differences between the vase life period of Carnation flowers which infested by *H. cottei* and *T. urticae* compared to non-infested flowers (control) at both the five examined varieties of Carnation. Whereas F (0.05) value and LSD value for the five examined varieties of Carnation were (345.43, 1.032), (276.87, 1.053), (264.21, 1.034) (341.93, 1.041) and (269.17, 1.067) respectively.

These results agree with those obtained by Mirab, M. (2015) in Iran who reported that were several species of Haplothrips are associated with flowers of Carnation plants and some other ornamental plants, and they cause serious damages to the stage of the flower. Jaskiewicz, B. (2008) in Poland who reported the effect of the Carnation thrips, *H. cottei* feeding on the flowering of Carnation and reported that *H. cottei*, when found in greater numbers, caused deformation of the leaf blades, shorting of shoots and petioles, as well as deformation of the flowers. Miles, A. (2015) in Australia reported that in warm weather, *T. urticae* walks off buds of Carnation during a "critical period " coinciding with the opening of the sepals, and studies showed this behavior of pest feeding affected on the vase life period of these flowers after picking. Also, results obtained agreement with those obtained by Stone, M. (2012) who studied effect of infested Carnation flowers by three species of thrips on the vase life period of these flowers and estimated the damage on these flowers as a result of infestation by these insects.

Effect of Insect Infestation by *H. cottei* and *T. urticae* on the Internal Components of Carnation Flowers:

1- Effect of Insect Infestation by *H. cottei* and *T. urticae* on Total Soluable Sugare:

Data tabulated in Table (2) show the total soluble sugar content in different varieties (colors) of Carnation flowers after infestation by *H. cottei* and *T. urticae* compared to control.

Whereas total soluble sugar content at the five varieties of Carnation flowers (yellow, red, pink, blue and white) which infested by *H. cottei* were 26.42, 24.18, 22.65, 20.32 and 22.67 (mg/g) respectively, total soluble sugar content at the five varieties of Carnation flowers which infested by *T. urticae* were 30.32, 28.54, 25.71, 23.45 and 25.38 (mg/g) respectively, while total soluble sugar content at the five varieties of Carnation flowers in control which not infested by any pests were 35.26, 33.18, 31.21, 28.57 and 32.45 (mg/g) respectively.

Table (2): Determination of total soluble sugar (mg/g) in different colors of Carnation flowers infested by *H. cottei* and *T. urticae* compared to control

Color	Determination of total soluble sugar (mg/g)				
	H. cottei	*T. urticae*	Control	F(0.05)	LSD
Yellow	26.42^{c}	30.32^{b}	35.26^{a}	425.53	1.023
Red	24.18^{b}	28.54^{c}	33.18^{a}	543.87	1.034
Pink	22.65^{c}	25.71^{b}	31.21^{a}	485.62	1.082
Blue	20.32^{c}	23.45^{b}	28.57^{a}	347.91	1.098
White	22.67^{b}	25.38^{c}	32.45^{a}	456.82	1.076

Means within columns bearing different subscripts are significantly different ($P< 0.05$)

Generally, the infestation by *H. cottei* reduced total soluble sugar in all varieties of Carnation flowers more than the infestation by *T. urticae* compared to control.

Statistical analysis in (Table 2) show high significant differences between the total soluble sugar in different Carnation varieties which infested by *H. cottei* and *T. urticae* compared to control, whereas F(0.05) value & LSD value for the five examined varieties of Carnation were (425.53, 1.023), (543.87, 1.034), (485.62, 1.082), (347.91, 1.098) and (456.82, 1.076) respectively.

Effect of Insect Infestation by *H. cottei* and *T. urticae* on Total Protein:

Data tabulated in Table (3) show the total protein content in different varieties (colors) of Carnation flowers after infestation by *H. cottei* and *T. urticae* compared to control. Whereas total protein content at the five varieties of Carnation flowers (yellow, red, pink, blue and white) which infested by *H. cottei* were 17.42, 14.18, 12.65, 19.32 and 17.87 (mg/g) respectively, total protein content at the five varieties of Carnation flowers which infested by *T. urticae* were 21.25, 19.61, 17.83, 22.54 and 21.65 (mg/g) respectively, while total protein content at the five varieties of Carnation flowers in control which not infested by any pests were 27.56, 25.68, 23.21, 28.57 and 26.54 (mg/g) respectively.

Table (3): Determination of total protein (mg/g) in different colors of Carnation flowers infested by *H. cottei* and *T. urticae* compared to control

Color	Determination of total protein (mg/g)				
	H. cottei	*T. urticae*	Control	F(0.05)	LSD
Yellow	17.42^{c}	21.25^{c}	27.56^{a}	245.53	1.023
Red	14.18^{c}	19.61^{b}	25.68^{a}	341.65	1.043
Pink	12.65^{b}	17.83^{b}	23.21^{a}	365.78	1.082
Blue	19.32^{c}	22.54^{c}	28.57^{a}	234.97	1.067
White	17.87^{c}	21.65^{b}	26.54^{a}	375.92	1.034

Means within columns bearing different subscripts are significantly different ($P< 0.05$)

Generally, the infestation by *H. cottei* reduced total protein in all varieties of Carnation flowers more than the infestation by *T. urticae* compared to control.

Statistical analysis in (Table 3) show high significant differences between the total

proteins in different Carnation varieties which infested by *H. cottei* and *T. urticae* compared to control whereas F(0.05) value & LSD value for the five examined varieties of Carnation were (245.53, 1.023), (341.65, 1.043), (365.78, 1.082), (234.97, 1.067) and (375.92, 1.034) respectively.

Change in Protein Banding Patterns:

Data tabulated in table (4) Show the changes in protein banding patterns (amino acids) of infested Carnation flower petals by *H. cottei* and *T. urticae* compared to control (non-infested flowers). And showed that the infestation by *H. cottei* and *T. urticae* affected the number and arrangement of the protein banding patterns (amino acids) of infested Carnation flowers.

The obtained results are agreement with those obtained by Galeotti, F. *et al.* (2008)The secondwho studied the effect of *H. cottei* on the interior components of Carnation flowers, he found that the total protein in the Carnation petals reduced as result to the infestation by *H. cottei.* Peng, Z. and Miles, P. (2007) studied the changes in the internal components of Carnation flowers such as protein, sugar and vitamins, which infested by two species of thrips,

Table (4): Change induced by infestation with *H. cottei* and *T. urticae* in the protein banding pattern (amino acids) of Carnation flowers.

No of band	M.wt. (kDa)	Marker (M)	Control	*H. cottei*	*T. urticae*
1	199.0	Glycen	-	-	-
2	115.0	Alanen	+	-	+
3	89.0	Valen	+	-	+
4	77.0	Liocen	+	-	-
5	65.0	Isoliocen	+	-	-
6	51.0	Brolen	+	+	+
7	44.0	Venilalanen	+	+	+
8	31.9	Treptovan	-	+	-
9	33.0	Methionen	+	-	+
10	31.0	Aspartek acid	+	+	+
11	30.7	Glotamic acid	+	-	+
12	25.0	Laycen	+	-	-
13	22.0	Argnen	-	+	+
14	25.4	Hesteden	+	+	+
15	19.9	Seren	+	-	-
16	12.7	Sestayn	+	+	+
17	11.14	Asparagen	+	+	-
18	11.2	Glotamen	+	+	+
Total	-	18	15	9	11

M.wt. : Molecular weights
kDa : Kilo Dalton

Carnation thrips (*H. cottei*) and *Franklinella tritici*. Becker, W. and Apel, K. (2016) reported that the decrease in total protein may be due to the decrease in carbohydrate content which acts as a carbon source in protein synthesis in Carnation flowers due to the infestation by *T. urticae.* Atwal, A. and Dhingra, S. (2008) reported that the infestation by *H. cottei* was changed in the protein pattern in the Carnation petals.

Also, the obtained results are an agreement with those obtained by Nichols,

R. (2010) in France who studied the quantitative changes in soluble sugars (glucose, fructose, and sucrose) of Carnation petals as a result of infestation by three species of thrips and estimated the damage. Decheva, R. *et al*. (2001) in Bulgaria investigated the changes in the total sugar (glucose, fructose, and sucrose), starch, free amino acid, and protein in buds of Carnation flowers, the level of 12 free amino acids identified decreased as result of the infestation by two species of thrips.

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ARABIC SUMMARY

أثر إصابة زهور القرنفل ب *Tetranychus urticae* و *Haplothrips cottei* على طول فترة حياة الزهور بعد القطف (Vase life period) تحت ظروف الصوب الزجاجية

أشرف صلاح إمام ، خالد عبد العزيز عياد و عادل أمين محمد عبد الله
معهد بحوث وقاية النباتات - مركز البحوث الزراعية – الدقى – الجيزة – مصر

أجريت هذه الدراسة بغرض دراسة أثر إصابة زهورالقرنفل بتربس القرنفل *Haplothrips cottei* والعنكبوت الأحمر *Tetranychus urticae* على طول فترة حياة الزهور بعد القطف (Vase life period) وذلك تحت ظروف الصوب الزجاجية . أجريت هذه الدراسة فى موقعين مختلفين هما الحديقة الدولية (محافظة القاهرة) وحديقة الأورمان (محافظة الجيزة) خلال عام 2018. وانقسمت هذه الدراسة إلى جزئين أساسين :

الجزء الأول: دراسة أثرإصابة زهور القرنفل بتربس القرنفل *H. cottei* و العنكبوت الأحمر *T. urticae*على طول فترة حياة الزهور بعد القطف (Vase life period) وتوصلت النتائج إلى وجود تأثير واضح للإصابة بالافتين موضع الدراسة على طول فترة حياة أزهار القرنفل بعد القطف وذلك بالمقارنة بأزهار القرنفل الغير مصابة (الكنترول) . كما أوضحت النتائج زيادة تأثير الإصابة بحشرة *H. cottei* عن تأثير الإصابة ب *T. urticae* على تناقص طول فترة حياة أزهار القرنفل بعد القطف وذلك مقارنة بأزهار القرنفل الغير مصابة (الكنترول) .

الجزء الثانى: دراسة أثر الاصابة بكلتا الافتين *T. urticae* , *H. cottei* على المكونات الداخلية لأزهار القرنفل والتى لها علاقة وثيقة بطول فترة حياة الأزهار بعد القطف مثل إجمالى السكريات وإجمالى البروتين. وتوصلت النتائج إلى تزايد تأثير الإصابة بحشرة *H. cottei* على المجموع الكلى للسكريات وكذلك البروتين الموجود فى أزهار القرنفل المصابة بدرجة أكبر من الإصابة بالعنكبوت الأحمر *T. urticae* وذلك بالمقارنة بأزهار القرنفل الغير مصابة (الكنترول) . كما أوضحت النتائج وجود تأثير للإصابة بكلتا الأفتين على عدد وترتيب الأحماض الأمينية المشكلة للبروتين الموجود داخل بتلات أزهار القرنفل وذلك بالمقارنة بأزهار القرنفل الغير مصابة.