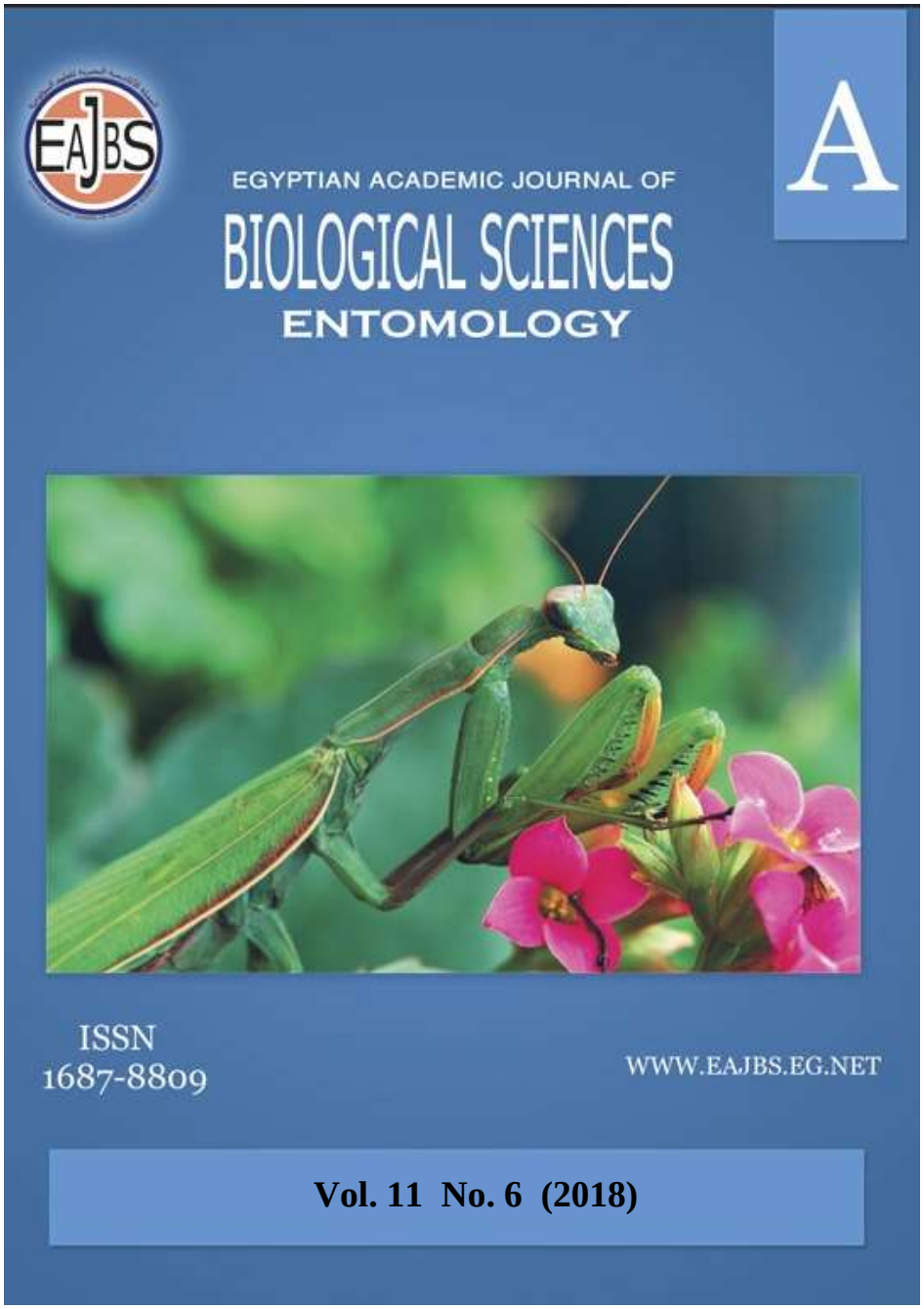

Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.
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www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 11(6)pp: 1- 9 (2018)*

Egypt. Acad. J. Biolog. Sci., 11(6):1– 9 (2018)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
www.eajbs.eg.net

Effect of Insect Infestation by *Macrosiphum rosae* L. on the Vase Live Period of Rose Flowers under Greenhouse Conditions

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ARTICLE INFO
Article History
Received:1/9/2018
Accepted:3/10/2018

Keywords:
Macrosiphum rosae L.. rose flowers, greenhouse conditions

ABSTRACT

This study was carried out to study the effect of insect infestation by *Macrosiphum rosae* L. on the vase life period on the rose flowers under greenhouse conditions at two locations International Garden (Cairo Governorate) and El-Orman Garden, (Giza Governorate) under glasshouse conditions during successive seasons 2018. This is because vase life period is a 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 the effect of insect infestation by *M. rosae* on the vase life period of rose flowers after picking. Second part studied the effect of insect infestation by the same insect on the internal components of the rose flowers which correlated with vase life period of these flowers such as total sugar and total protein. Results showed that the infestation by *M. rosae* reduced the vase life period of rose flowers after picking at the infested rose flowers compared to control (which non infested by the same insect). Also, results showed that the infestation by *M. rosae* reduced total sugar and total protein at the infested rose flowers compared to control. Lastly, results showed that the infestation by *M. rosae* changed the number and arrangement of the protein banding patterns (amino acids) of infested rose flowers petals compared to control.

INTRODUCTION

Rose (*Rosa gallica*) 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 newly reclaimed areas for purposes local consumption and exportation to the foreign markets. Rose named king of flowers because it found from oldest countries and it is the favorite flower for human all over the world. Although developing live and high technical but love human to rose still and increase. The human love to the roses due to their beautiful colors, style of flowers, smiles, and tolerant the inferable weather factors. Later rose became one of the important components for international income for many countries all over the world through exporting these roses to the different countries, Emam (2009).

Rose infested with the large scale of insects belong to many orders and families such as aphids, as an important group of insects which are belonged to order Hemiptera. *Macrosiphum rosae* L., commonly known as rose aphid, is an important

pest of rose and many other crops. Jaskiewicz (1997) who reported that the strong infestation by the rose aphid, *M. rosae* resulted in the deformation of stems, leaves and flowers. Derek (1977) in Australia who reported that *M. rosae* is a serious pest on rose, and it is reproducing, parthenogenetically and viviparously all year round. It feeds mainly on the young leaves and developing flower-buds of roses.

The aim of this work is studied effect of insect infestation by *M. rosae* on the vase life period of rose flowers at two locations, International Garden (Cairo Governorate) and El-Orman Garden, (Giza Governorate) under glasshouse conditions during successive seasons 2018. This is because vase life period is a 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 study the effect of insect infestation by *M. rosae* on the vase life period of rose flowers after picking. The second part studies the effect of insect infestation by the same insect on the internal components of the rose flowers which correlated with vase life period of these flowers such as total sugar and total protein.

MATERIALS AND METHODS

Experimental Design:

This study was conducted on rose plants grown in two locations, International Garden (Cairo Governorate) and El-Orman Garden, (Giza Governorate) under glasshouse conditions during successive seasons 2018. Two glasshouses in each garden with an area of 27x45 m of each one, was divided into 4 plots (3x5 m^2), the first one of these glasshouses contained infested rose plants and other one as the control. The first glasshouse was arranged in randomized block with four replicates to four varieties (colors) of rose, and also the second glasshouse was arranged in randomized block with four replicates as the control. The 1st glasshouse was artificially infestation by *M. rosae* and the 2nd one was left as a control. The two glasshouses were in an area isolated from other trees in the garden. Also, the first glasshouse was isolated from the second one. Rose plants were planted in glasshouse conditions at the same time on November (the planting time of rose 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 insect *M. rosae* in the first glasshouse, with careful observation of the mean numbers of *M. rosae* during plant growth period and especially during the flowering stage from February – August with examining the second glasshouse (control). At the end of the first growing season, 100 flowers were collected from the 1st glasshouse and 100 other flowers from the 2ndglasshouse at the two locations. In 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. With 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 *M. rosae* Infestation on the Internal Components of Rose Flowers:

These experiments were carried out to study the effect of insect infestation by *M. rosae* on the vase life period of rose flowers through study the effect of insect infestation by the same insect on the internal components of rose flowers specifically two elements (sugars and 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 fresh tissue. 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% bromophenolbule 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 marker.

Electrophoresis Conditions:

The running buffer was poured into 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 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 reaches the bottom of the resolving gel.

Gel Staining and Distaining:

After the completion of the run, the gel was placed in 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 distained with 200ml destaining solution (100ml glacial acetic acid, 400ml methanol and completed to 1L by distilled water) and agitated gently on the shaker. The distaining 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 rose aphid *M. rosae* on the vase life period of the rose flowers. And the effect of the infestation by the same insect on the total soluble sugar and total protein of the rose 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 the effect of insect infestation by *M. rosae* on the vase life period of the rose flowers at two locations, International Garden (Cairo Governorate) and El-Orman Garden, (Giza Governorate) under glasshouse conditions during successive seasons 2018. This is because vase life period is a 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 the effect of insect infestation by *M. rosae* on the vase life period of rose flowers after picking. Second part studied the effect of insect infestation by the same insect on the internal components of the rose flowers which correlated with vase life period of these flowers such as total sugar and total protein.

1-Effect of the Insect Infestation by Rose Aphid, *M. rosae* on the Vase Life Period of Rose Flowers:

This experiment was carried out to study the effect of the insect infestation by rose aphid *M. rosae* on the vase life period of rose flowers. And means of vase life period (flowers life after picking) for infested flowers and control (non infested) at the two examined locations were recorded.

Results in Table (1) show mean lifetime (vase life period) of the rose flowers which infested by *M. rosae* compared to control (non infested) for

Table (1): Effect of insect infestation by *M. rosae* on the vase life period of rose flowers after picking compared to control.

Rose	Vase life period per days	
	Infested	Control
Yellow	3.9^{a}	9.7^{a}
Red	4.7^{b}	10.3^{b}
Pink	5.4^{c}	11.4^{c}
White	6.3^{d}	12.5^{d}
F	**833.03**	**65.53**
LSD	**1.0213**	**4.7232**

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

The four varieties (colors) of rose (yellow, red, pink and white), respectively at the two examined locations. Whereas the mean of vase life period of rose flowers for both the four varieties of rose were 3.9, 4.7, 5.4 and 6.3 days, respectively. Compared to control which mean of the vase life period were 9.7, 10.3, 11.4 and 12.5 days for the four varieties respectively.

Statically analysis shows highly significant differences between the vase life period of rose flowers which infested by *M. rosae* compared to non infested flowers (control) at both the four varieties (colors) of rose. Whereas LSD values were (1.02 and 4.72) for the infested and non infested rose, respectively.

These results agree with those obtained by Emam (2009) who reported that the adults and nymphs of rose aphid *M. rosae* attack the rose plants and suck cell sap from flowers, tender shoots and buds, ultimately decreasing the market value of rose flowers. Aphid infestation badly affects the flowering capacity of plants, resulting in

20-40% losses. The aphids are apterous and reproduce parthenogenetically and also aphid populations may increase very rapidly under natural conditions. Jaskiewicz (2006) in Poland who reported the effect of the rose aphid *M. rosae* feeding on the flowering of roses and reported that aphid *M. rosae* when found in greater numbers caused deformation of the leaf blades, shorting of shoots and petioles, as well as deformation of the flowers. Miles (1985) in Australia reported that in warm weather , the *M. rosae* walks off buds of roses during a "critical period " coinciding with the opening of the sepals , and studies showed this behavior of insect feeding affected on the vase life period of these flowers after picking.

2-Effect of Insect Infestation by *M. rosae* on the Internal Components of Rose Flowers which Correlated with Vase Life Period:

- Effect of Insect Infestation by *M. rosae* on Total Soluble Sugar:

Data in Table (2) show the total soluble sugar content in different varieties (colors) of rose flowers after infestation by rose aphid *M. rosae* compared to control. Whereas the total soluble sugar content at the four varieties of rose flowers (yellow, red, pink and white) were 27.42, 24.18, 22.65 and 18.32 (mg/g) respectively, while these values were 37.56, 35.68, 33.21 and 28.57 (mg/g) in control, respectively.

Generally, the infestation by rose aphid *M. rosae* reduced total soluble sugar in all varieties of rose flowers compared to control.

Statistical analysis in (Table 2) shows highly significant differences between the total soluble sugar in different rose colors which infested by rose aphid *M. rosae* compared to control whereas F value = 745.53 and 89.67 & L.S.D. (0.05)= 1.0235 and 5.6384 for the infested and non infested rose varieties respectively.

Tabl(2): Determination of total soluble sugar (mg/g) in different colors of rose flowers after infestation by rose aphid, *M. rosae*

Color	Determination of total soluble sugar (mg/g)	
	M. rosae	**Control**
Yellow	27.42^{a}	37.56^{a}
Red	24.18^{b}	35.68^{b}
Pink	22.65^{c}	33.21^{c}
White	18.32^{d}	28.57^{d}
F	**745.53**	**89.67**
LSD	**1.0235**	**5.6384**

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

-- Effect of Insect Infestation by *M. rosae* on Total Protein:

Data in Table (3) show the total protein content in different varieties (colors) of rose flowers after infestation by rose aphid *M. rosae* compared to control. Whereas the total protein content at the four varieties of rose flowers (yellow, red, pink and white) were 38.32, 31.45, 27.35 and 20.42 (mg/g) respectively, while these values were 49.67, 42.33, 39.45 and 31.74 (mg/g) in control, respectively.

Generally, the infestation by rose aphid *M. rosae* reduced total protein in all varieties of rose flowers compared to control.

Statistical analysis in (Table 3) shows highly significant differences between the total protein in different rose colors which infested by rose aphid *M. rosae* compared to control whereas F value = 341.65 and 95.78 & L.S.D. (0.05)= 2.3245 and 4.1375 for the infested and non infested rose varieties respectively.

Table (3): Determination of total protein (mg/g) in different colors of rose flowers after infestation by rose aphid, *M. rosae*

Color	Determination of total protein per (mg/g)	
	M. rosae	**Control**
Yellow	38.32^{a}	49.67^{a}
Red	31.45^{b}	42.33^{b}
Pink	27.35^{c}	39.45^{c}
White	20.42^{d}	31.74^{d}
F	**341.65**	**95.78**
LSD	**2.3245**	**4.1375**

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

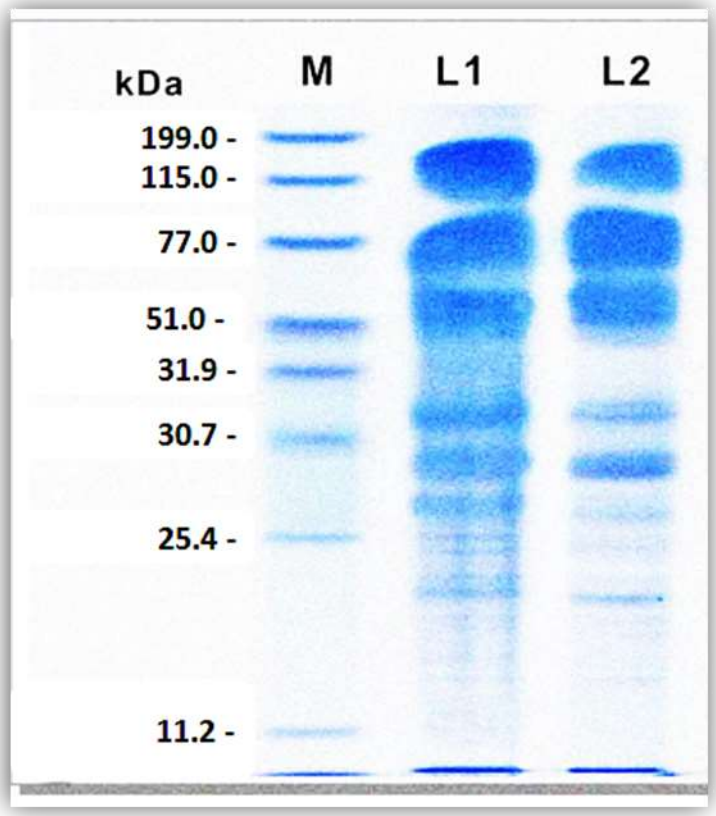
Change in Protein Banding Patterns:

Data in the table (4) and Fig. (1) Show the changes in protein banding patterns (amino acids) of infested rose flowers petals by *M. rosae* compared to control (non infested flowers). And showed that the infestation by *M. rosae* affected on the number and arrangement of the protein banding patterns (amino acids) of infested rose flowers petals.

Table (4) indicate the occurrence of ten common protein bands appeared in the petals of control and in petals response to the applied infestation by rose aphid (M.wt: 115.0, 77.0, 65.0, 44.0, 33.0, 30.7, 25.0, 25.4, 12.7 and 11.14 kDa). Three different polypeptides with molecular weights (199.0, 31.9 and 22.0 kDa) were not detected as common bands in petals in response to infestation by aphid and in the control. Five different polypeptides with molecular weights (89.0, 51.0, 31.0, 19.9 and 11.2 kDa) were detected as common bands in control and not detected in the infested petals.

Table (4): Change induced by infestation with rose aphid, *M. rosae* in the protein banding pattern (amino acids) of rose flowers.

No of band	M.wt. (kDa)	Marker (M)	Control	Aphid
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 No of bands	-	18	15	10



M.wt. : Molecular weights kDa : Kilo Dalton
M= Marker L1 : Control L2 : Aphid

Fig. (1): Change in protein banding patterns (amino acids) of rose flowers petals in response to rose aphid, *M. rosae* infestation.

The obtained results are in agreement who those obtained by Emam (2009) in Egypt who studied the effect of *M. rosae* and *F. occidentalis* on the interior components of rose flowers. He reported that the total protein in the rose petals reduced as result to the infestation by *M. rosae.* Peng and Miles (1991) studied the changes in the internal components of rose plants such as protein, sugar and vitamins, which infested with some insects and decided that the most effective was by *M. rosae* and *F. tritici*. Becker and Apel (1992) 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 rose flowers due to the infestation by *M. rosae*. Atwal and Dhingra (2008) reported that the infestation by *M. rosae* was changed in the protein pattern in the rose petals. While, Jaskiewicz (2006) studied the changes which happened in protein pattern in the rose petals which infested by *M. rosae*.

Also, the obtained results are in agreement with those obtained by Pankovetskii and Tyutyunnik (1978) in Russia who determined starch and sugar contents in the leaves , sepals and petals of the rose varieties during bud formation and flowering . They found that during bud formation the starch content rose in all organs but most of all in the petals (up to 30%). During flowering starch content decreased in the petals , sepals and leaves by 72 – 96 , 56 – 80 and 40 – 60 % respectively , and the sugar content rose rapidly in the petals, Decheva *et al.* (1986) in Bulgaria investigated the changes in the sugar, starch, free amino acid and protein

from August to March in buds of flowers of rose plants , total sugar (glucose , fructose , and sucrose) content varied until February and was maximal before bud break . The level of the 12 free amino acids identified decreased during dormancy, and then rose rapidly at bud swelling in March. In the dry weather, sugar, starch and free amino acid contents were higher in buds of the flower-bearing plants, Peng and Miles (1988) in Australia stated that tissue sap of rose became more acceptable to the aphids *M. rosae* as a result of oxidation. When the rose aphid fed on stems of semi-dormant miniature rose bushes, catechin and protein content was reduced in the immediate vicinity of colonies, but rose temporarily to higher than normal levels after the insects had been removed. When the roses were growing vigorously, however, rapid changes in tissue chemistry tended to mask this interaction,

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ARABIC SUMMERY

أثر الاصابة الحشرية بحشرة من الورد *Macrosiphum rosae* L. على طول فترة بقاء الأزهار بعد القطف (Vase life) لأزهار الورد تحت ظروف الصوب الزجاجية

أشرف صلاح إمام و فرحه حسنى حسن فرج الله
معهد بحوث وقاية النباتات - مركز البحوث الزراعية – الدقى – الجيزة – مصر

أجريت هذه الدراسة بغرض دراسة أثر الاصابة الحشرية بحشرة من الورد *Macrosiphum rosae* L. على طول فترة بقاء الأزهار بعد القطف (Vase life) وذلك تحت ظروف الصوب الزجاجية . أجريت هذه الدراسة فى موقعين مختلفين هما الحديقة الدولية (محافظة القاهرة) وحديقة الأورمان (محافظة الجيزة) خلال عام ٢٠١٨. وانقسمت هذه الدراسة الى جزئين أساسين :

الأول هو دراسة أثر الاصابة الحشرية بحشرة من الورد *Macrosiphum rosae* L. على طول فترة بقاء الأزهار بعد القطف (Vase life) وتوصلت النتائج الى وجود تأثير واضح للاصابة بحشرة من الورد على طول فترة بقاء الأزهار بعد القطف حيث أوضحت النتائج تناقص طول هذه الفترة فى أزهار الورد المصابة بالحشرة وذلك مقارنة بأزهار الورد غير المصابة .

الجزء الثانى: دراسة أثر الاصابة بحشرة من الورد *M. rosae* على المكونات الداخلية لأزهار الورد والتى لها علاقة وثيقة بطول فترة بقاء الأزهار بعد القطف مثل السكريات و البروتين. وتوصلت النتائج الى وجود تأثير واضح للاصابة بحشرة من الورد على المجموع الكلى للسكريات و كذلك البروتين الموجود فى أزهار الورد المصابة بالحشرة وذلك مقارنة بأزهار الورد غير المصابة. كما أوضحت النتائج وجود تأثير للإصابة بذات الحشرة على عدد وترتيب الأحماض الأمينية المشكلة للبروتين الموجود داخل بتلات أزهار الورد وذلك مقارنة بأزهار الورد الغير مصابة.