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Effect of the infestation by *Aphis nerii* and *Tetranychus urticae* on the vase live period of jasmine flowers under glasshouse

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Abstract:

This study was carried out to study effect of infested jasmine flowers (*Jasmine* spp. Family Oleaceae) by *Aphis nerii* (Boyer) (Hemiptera:Aphididae) and *Tetranychus urticae* Koch. (Acari: Tetranychidae) on the vase life period (the flowers life after picking) of jasmine flowers under glasshouse conditions at two locations, El-Orman Garden (Giza Governorate) and International Garden (Alexandria Governorate) during successive season 2018. This is because vase life period is very important parameter in cut flowers and there are many factors affected on it. Therefore this study divided into two parts, first part studied effect of infested jasmine flowers by *A. nerii* and *T.urticae* on the vase life period of jasmine flowers after picking. Second part studied effect of infested jasmine 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 showed that the infestation by *A. nerii* reduced the vase life period of jasmine 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 *A. Nerii* reduced total suger and total protein at the infested jasmine flowers more than the infestation by *T.urticae* compared to control. Lastly, results showed that the infestation by *A. nerii* and *T.urticae* changed the number and arrange of the protein banding patterns (amino acids) of infested jasmine flowers petals compared to control.

Introduction

Jasmine flowers (*Jasmine* spp. Fam. Oleaceae) is considered one of the most important and popular cut flowers in Egypt and allover the world which cultivated in the open field and under greenhouse conditions. A jasmine flower is a very popular flower around the world especially in the tropics because of its unique fragrance. 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, this beside its uses in different medicinal purposes.The human love to the jasmine flowers due to their beautiful colors, style of flowers, smiles

and tolerant the inferable weather factors (Fishman *et al*. , 2015).

Jasmine flowers infested with large scale of insects belong to many orders and families such as *Aphis nerii* (Boyer) (Hemiptera: Aphididae) and *Tetranychus urticae* Koch. (Acari: Tetranychidae) which are consider important pests of jasmine flowers and many other flowers. Jaskiewicz (2012) who reported that the strong infestation by *A.nerii* resulted in the deformation of stems, leaves and flowers of jasmine plants. Derek (2015) in Australia who reported that *A. nerii* and *T. urticae* are serious pests on jasmine flowers, andthey feed mainly on the young leaves and developing flower-buds of jasmine flowers.

This study was carried out to study the effect of infested jasmine flowers by *A.nerii* and *T.urticae* on the vase life period of jasmine flowers under glasshouse conditions at two locations, El-Orman Garden (Giza Governorate) and International Garden (Alexandria Governorate) during successive season 2018.

Materials and methods

1. Experimental design:

This study was conducted on jasmine flowers grown in two locations El-Orman Garden (Giza Governorate) and International Garden (Alexandria 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 artificially infestation by *A. nerii* and the third part had artificially infestation by *T. urticae*. Each part contains 5 plots (3x5 m^2) for each, and each part isolated completely from others. Jasmine seedlings were planted in glasshouse conditions at the same time on November (the planting time of jasmine 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 *A.nerii* in the second part and by *T.urticae* in the third part with careful observation of the mean numbers of these pests during 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 postharvest 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 flower 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.

2. Effect ofinfestation by *Aphis nerii* and *Tetranychus urticae* on the internal components of jasmine flowers:

These experiments was carried out to study effect of infestation by *A.nerii* and *T.urticae* on the vase life period of jasmine flowers thourough study effect of the infestation by the same pests on the internal components of jasmine flowers specifically two elements (total sugars and total protein) which have strong correlated with the vase life period.

3. Determination of protein banding pattern:

3.1. Total protein extraction:

Total proteins were extracted from 0.5 kg fresh tissue of jasmine 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).**

3.2. Loading on a gel:

3.2.1. 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 hour and finally 150v for the remaining 1.5hour. **(Sheri *et al*., 2000).**

3.2.2.Sample loading:

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

3.2.3.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 reachs the bottom of the resolving gel.

3.2.4.Gel Staining and distaining:

After the completion of the run, gel was placed in staining solution consisting of 1g of Coomassie Brilliant bule–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.

3.2.5.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.

4.Statistical analysis:

In the experiments,effect of the infestation by *A.nerii* and*T.urticae* on the vase life period of the jasmine flowers and effect of the infestation by the same pests on the total soluble sugar and total protein of the Jasmine 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 Chouromatograph (HPLC).

Results and discussion

1. Effect of the infestation by *Aphis nerii* and*Tetranychus urticae* on the vase life period of jasmine flowers:

Data tabulated in Table (1) show means of life time (vase life period) of the jasmine flowers which infested by *A. nerii* and *T. urticae* compared to control (the flowers non infested) for the five varieties (colors) of jasmine flowers (yellow, red, pink, blue and white) at the two examined locations. Data obtained showed the means of vase life period of jasmine flowers in control which did not infested by any pests ranged from 9.7 to 12.3 days for the five varieties (colors) of jasmine flowers, means of vase life period of jasmine flowers which infested by*A. nerii* ranged from 4.9 to 6.8 days, while means of vase life period of jasmine flowers which infested by *T. urticae* ranged from 5.3 to 7.8 days.

Table (1): Effect of insect infestation by *Aphis nerii* and *Tetranychus urticae* on the vase life period of jasmine flowers after picking compared to control.

Jasmine	Vase life period per days				
	Aphis nerii	***Tetranychus urticae***	**Control**	**F(0.05)**	**LSD**
Yellow	6.3^{c}	7.2^{b}	11.5^{a}	215.44	1.052
Red	6.8^{c}	7.5^{b}	10.3^{a}	283.17	1.043
Pink	5.3^{b}	6.5^{c}	9.7^{a}	334.21	1.024
Blue	4.9^{c}	5.3^{c}	10.2^{a}	251.96	1.052
White	5.9^{c}	7.8^{b}	12.3^{a}	253.15	1.037

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

Statistical analysis showed that high significant differences between the vase life period of jasmine flowers which infested by *A. nerii* and *T. urticae* compared to non-infested flowers (control) at both the five examined varieties of jasmine flowers. Whereas F (0.05) value and LSD value for the five examined varieties of jasmine were (215.44, 1.052), (283.17, 1.043), (334.21, 1.024) (251.96, 1.052) and (253.15, 1.037), respectively.

These results agreement with those obtained by Jaskiewicz (2012) in Poland who reported the effect of *A. nerii* feeding on the flowering of jasmine and reported that *A. nerii* when found in greater numbers caused deformation of the leaf blades, shorting of shoots and petioles, as well as deformation of the flowers. Miles (2015) in Australia reported that in warm weather *T. urticae* walks off buds of jasmine during a "critical period " coinciding with the opening of the sepals, and studied 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 (2012) who studied effect of infested jasmine flowers by thouree species of aphids on the vase life period of these flowers and estimated the damage on these flowers as a result of infestation by these insects.

2. Effect of the insect infestation by *Aphis nerii* and *Tetranychus urticae* on the internal components of jasmine flowers:

2.1. Effect of the insect infestation by*Aphis nerii* and *Tetranychus urticae* on the total soluable sugar:

Data tabulated in Table (2) show the total soluble sugar content in different varieties (colors) of jasmine flowers after infestation by *A. nerii* and *T. urticae* compared to control. Whereas total soluble sugar content at the five varieties of jasmine flowers (yellow, red, pink, blue and white) which infested by *A. nerii* were 23.52, 25.16, 23.45, 21.52 and 22.37 (mg/g) respectively, total soluble sugar content at the five varieties of jasmine flowers which infested by *T. urticae* were 29.32, 30.54, 29.75, 27.15 and 26.32 (mg/g), respectively,while total soluble sugar content at the five varietiesof jasmine flowers in control which non infested by any pests were 33.25, 34.28, 32.25, 30.57 and 31.25 (mg/g), respectively. Generally, the infestation by *A. nerii* reduced total soluble sugar in all varieties of jasmine flowers more than the infestation by *T. urticae* compared to control. Statistical analysis in (Table, 2) showed high significant differences between the total soluble sugar in different jasmine varieties which infested by *A. nerii* and *T. urticae* compared to control, whereas F(0.05) value and LSD value for the five examined varieties of jasmine were (325.32, 1.045), (243.45, 1.034), (325.52, 1.052), (234.21, 1.032) and (352.15, 1.025), respectively.

Table (2): Determination of total soluble sugar (mg/g) in different colors of jasmine flowers infested by*Aphis nerii* and *Tetranychus urticae* compared to control.

Color	Determination of total soluble sugar (mg/g)				
	Aphis nerii	*Tetranychus urticae*	Control	F(0.05)	LSD
Yellow	23.52[c]	29.32 [b]	33.25[a]	325.32	1.045
Red	25.16[b]	30.54 [c]	34.28[a]	243.45	1.034
Pink	23.45[c]	29.75 [b]	32.25[a]	325.52	1.052
Blue	21.52[c]	27.15 [b]	30.57[a]	234.21	1.032
White	22.37 [b]	26.32 [b]	31.25 [a]	352.15	1.025

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

2.2. Effect of insect infestation by *Aphis nerii* and *Tetranychus urticae* on total protein:

Data tabulated in Table (3) showed the total protein content in different varieties (colors) of jasmine flowers after infestation by *A. nerii* and *T. urticae* compared to control. Whereas total protein content at the five varieties of jasmine flowers (yellow, red, pink, blue and white) which infested by *A.nerii* were 16.32, 15.28, 13.45, 18.72 and 17.25 (mg/g), respectively, total protein content at the five varieties of jasmine flowers which infested by *T. urticae* were 20.15, 19.31, 18.23, 21.14 and 20.35 (mg/g), respectively, while total protein content at the five varieties of jasmine flowers in control which non infested by any pests were 26.35, 27.42, 24.11, 27.35 and 26.44 (mg/g), respectively. Generally, the infestation by *A. nerii* reduced total protein in all varieties of jasmine flowers more than the infestation by *T. urticae* compared to control.

Table (3): Determination of total protein (mg/g) in different colors of jasmine flowers infested by *Aphis nerii* and *Tetranychus urticae* compared to control.

Color	Determination of total protein (mg/g)				
	Aphis nerii	*Tetranychus urticae*	Control	F(0.05)	LSD
Yellow	16.32[c]	20.15 [c]	26.35[a]	265.33	1.033
Red	15.28[c]	19.31 [b]	27.42[a]	322.15	1.052
Pink	13.45[b]	18.23 [b]	24.11[a]	215.28	1.032
Blue	18.72[c]	21.14 [c]	27.35[a]	234.15	1.044
White	17.25 [c]	20.35 [b]	26.44 [a]	370.12	1.035

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

Statistical analysis in (Table, 3) show high significant differences between the total proteins in different jasmine varieties which infested by *A. nerii* and *T. urticae* compared to control whereas F(0.05) value and LSD value for the five examined varieties of jasmine were (265.33, 1.033), (322.15, 1.052), (215.28, 1.032), (234.15, 1.044) and (370.12, 1.035), respectively.

2.3. Change in protein banding patterns:

Data tabulated in Table (4) showed the changes in protein banding patterns (amino acids) of infested jasmine flowers petals by *A. nerii* and *T. urticae* compared to control (non infested flowers). Also showed that the infestation by *A. nerii* and *T. urticae* affected on the number and arrange of the protein banding patterns (amino acids) of infested jasmine flowers.The obtained results are agreement with those obtained by Galeotti *et al.* (2014) who studied effect of the infestation by rose aphid *M. rosa e*on the interior components of jasmine flowers, they found that the total protein in the jasmine petals reduced as result to the infestation by aphid. Peng and Miles (2017) studied the changes in the internal components of jasmine flowers such as protein, sugar and vitamins which changed as result of infestation by aphid. Becker and Apel (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 jasmine flowers due to the infestation by *T. urticae.*

Table (4): Change induced by infestation with *Aphis nerii* and *Tetranychus urticae* in the protein banding pattern (amino acids) of jasmine flowers.

No of band	M.wt. (kDa)	Marker (M)	Control	*Aphis nerii*	*Tetranychus 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	14	8	10

M.wt. : Molecular weights kDa : Kilo Dalton

Also, the obtained results are agreement with those obtained by Nichols (2010) in France who studied the quantitative changes in soluble sugars (glucose, fructose and sucrose) of jasminepetals as a result of infestation by thouree species of aphids and estimated the damage. Decheva *et al.* (2011) in Bulgaria investigated the changes in thetotal sugar (glucose, fructose, and sucrose), starch, free amino acid and protein in buds of jasmine flowers, the level of 12 free amino acids identified decreased as result of the infestation by two species of aphids.

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