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Effect of Insect Infestation on some Internal Components of some Important Cactus Plants

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ABSTRACT

This study was carried out to study effect of insect infestation on some internal components of some important cactus plants. So this study was carried out to study effect of insect infestation by three important insects on the internal components of three important cactus plants, Egyption mealybug, *Icerya aegyptiaca* (Douglas) (Margarodidae: Homoptera) on *Aloe vera* (Fam. Liliaceae), Agave weevil, *Scyphophorus acupunctatus* L. (Curculionidae: Coleoptera) on *Agave americana* (Fam. Agavaceae) and Daphla aphid, *Aphis nerii* (Boyer) (Aphididae: Homoptera) on *Calotropis procera* (Fam. Asclepiadaceae). And this study was carried out at two locations (governorates), El-Orman Garden (Giza Governorate) and Antoniadis Garden (Alexandria Governorate) during 2018 season.Results obtained from this study refereed to reduce the concentration of the most internal components of theses cactus plants as result to infestation by these insects. And few internal components its concentration still did not change after infestation by these insects.Statically analysis showed highly significant differences between concentration of the most internal components of the infested cactus plants compared to concentration of the same components in the control (non infested cactus plants).

INTRODUCTION

Cactus plants consider one of the importance and famous ornamental plants all over the world. It is a large group of the anthropogenic plants most of them have many or few thorns. Water consists of about 95% of plant weight, this water is used by plants during drought. Cactus plants acquire their importance and fame due to its many kinds and shapes and ability to tolerate thirst and serve environmental and land conditions. This is in addition to the length of its life and does not require special service or maintenance, Ortega, P. *et al.* (2015)

Cactus plants are used for many purposes, including the purposes of decoration, coordination and beautification as nesting plants. This is due to the large diversity of colors, shapes and sizes of its flowers. This is in addition to being used for many medical purposes, as theses plants are an importance source for many pharmaceutical and cosmetic industries, Griffith, M. (2012)

This study included three important cactus plants, *Aloe vera* (Fam. Liliaceae), *Agave americana* (Fam. Agavaceae) and *Calotropis procera* (Fam. Asclepiadaceae)

1-*Aloe vera:*

A. vera is a succulent plant species of the genus Aloe, An evergreen perennial, it originates from the Arabian Peninsula but grows wild in tropical climates for agricultural and medicinal uses. *A. vera* is a popular medicinal plant that is used in the cosmetic, pharmaceutical and food industries. Its leaves are full of a gel- like substance that contains many beneficial compounds including vitamins, minerals, amino acids, Hamman, J. (2016). *A. vera* also contains various powerful antioxidant compounds. Some of these compounds can help inhibit the growth of harmful bacteria, Josias, H. (2008) found many of the health benefits associated with *A. vera* have been attributed to the polysaccharides contained in the gel of the leaves.

2-*Agave Americana*:

Agave is a genus of monocots native to the hot and regions of Mexico and Southwestern United States and other regions all over the world. It is an important economic product for its medicinal purposes. Leaves contain gel which has important components, most important of them are different steroid glycosides which has great important in therapy of the heart deceases. Gel also contains vitamins, sugars, fats and proteins which used to in different cosmetics for improve hair and skin, Souza, V. *et al.* (2013). Also Matthew, M. *et al.* (1999) reported that Agave Americana is a low growing, thick, long leaved, subtropical plant used for different medicinal, commercial and ornamental purposes, the plant's sap contains steroid glycosides, calcium oxalate crystals, acrid oils and other important compounds.

3-*Calotropis procera*:

C. procera is a shrub 2-4 m high stem white corky. Leaves obovate 15-20 cm. The used parts are bark, leaves, flowers and the latex. The essential and principle constituents are the cardinolide glycosides, it is used to treat heart diseases (Digitalis- like action on the heart) and regulation of heart rate De and Datt (1988) in India reported that *C. procera* is used to treat cardiovascular and heart diseases. Also, these glycosides used to adjust blood glucose. Beside these important glycosides *C. procera* plant also contains usharin, ushardin, calotropagenin, calctin and calotoxin. The plant contains also mandarin, gigantin, resins, alkaloids usharine and vorusharine and bitter principles. The unsaponifiable matter of the latex contains caoutchouc, trypsin, alpha 8 beta calotropeol, proteoclastic enzyme similar to papain. Bark contains two alcohols giganteol and iso giganteol. In India the plant is used locally for elephantiasis, leprosy and chronic enzema. Powdered flowers are useful in cases of cold, cough, asthma and indigestion. The plant is very toxic (15-20 times toxic as strychnine alkaloid), (Kumar, S. *et al*. 2001), (Sharma, p and Sharma, J. 2000).

These cactus plants infested by many different insects which greatly affect on their use in the purposes of decorative and also on the medical purposes.

There for these experiments were carried out to study effect of insect infestation by three important insects on the internal components of three important cactus plants, *Icerya aegyptiaca* (Douglas) on *A. vera*, *Scyphophorus acupunctatus* on *A. americana* and *Aphis nerii* (Boyer) on *C. procera* . And this study was carried out at two locations (governorates), El-Orman Garden (Giza Governorate) and Antoniadis Garden (Alexandria Governorate) during 2018 season.

MATERIALS AND METHODS

This study was carried out to study effect of insect infestation by three important insects on the internal components of three important cactus plants, Egyption mealybug, *Icerya aegyptiaca* (Douglas) on *Aloe vera*, Agave weevil, *Scyphophorus acupunctatus* on *Agave americana* and Daphla aphid, *Aphis nerii* (Boyer) on *Calotropis procera*. And this study was carried out at two locations (governorates), El-Orman Garden (Giza Governorate) and Antoniadis Garden (Alexandria Governorate) during 2018 season.

-Experimental design:

Samples contained six trees (replicates) from each variety of cactus in both the two gardens (governorates). Three infested trees and three uninfested trees for both of cactus plant through artificial infestation by these insects. And these trees were found in isolated aria at the two gardens. When these insects rich to its pick (highest infestation) on these cactus plants: October month for *I. aegyptiaca*, May month for *S. acupunctatus* and March month for *A. nerii* , took 15 leaves from the infested trees and 15 leaves from non infested trees to the lap at the two places. And took the means of concentrations of the important internal components of both infested and non infested plants.

-Determination of internal components of cactus plants:

Internal components extraction:

Internal components were extracted from 0.25 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).

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).

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.

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.

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, the sugar and protein were analyzed by High Pressure Liquid Chromatography (HPLC).

- Statistical analysis:

In these experiments, the effect of the insect infestation by certain insects on the internal components of certain cactus plants 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).

RESULTS AND DISCUSSION

This study was carried out to study effect of insect infestation by three important insects on the internal components of three important cactus plants, *Icerya aegyptiaca* (Douglas) on *Aloe vera*, *Scyphophorus acupunctatus* on *Agave americana* and *Aphis nerii* (Boyer) on *Calotropis procera* . And this study was carried out at two locations (governorates), El-Orman Garden (Giza Governorate) and Antoniadis Garden (Alexandria Governorate) during 2018 season.

1-Effect of I. *aegyptiaca* on *A. vera*:

Data tabulated in table (1) show the effect of insect infestation by *I. aegyptiaca* on the internal components of *A. vera* leaves. Whereas concentration of Vitamin A, Vitamin C, Vitamin E, Vitamin B12, Folic acid, Amino acids, Minerals, Sugars, Lignin and Beta carotene decreased from 2.75, 3.25, 1.75, 2.35, 3.45, 3.75, 2.75, 3.32, 1.75 and 2.53 mg/g, respectively to 1.98, 2.17, 1.15, 1.57, 2.33, 2.35, 1.82, 2.25, 0.95 and 1.25 mg/g, respectively. While concentration of Salicylic acids and Saponins did not change after infestation by *I. aegyptiaca*.

Statically analysis showed that were highly significant differences between concentrations of the most internal components of *A. vera* leaves which infested by *I. agyptiaca* compared to concentrations of the same components of non infested plants (control). Whereas F (0.05) value and L.S.D value were 234.85 and 1.76, respectively.

These results were agreement with those obtained by Gahukar, R. (2012) who found that the 69.8% reduction in Aloe vera plant damage refer to mealy bug feeding and also this feeding affecting highly on the components of the gel which found in their leaves. Ruparao, T. (2018) referred to the effect *Icerya purchase* attacking on the *Aloe vera* that losing about 10 percent of its production and this pest causes an economic injury

levels (EIL) and economic threshold levels (ETL), also this pest effect on the internal components of *Aloe vera* especially methanol leaf extract. Sofia, D. *et al*. (2017) in Bulgaria reported that *Icerya purchase* is the most dangerous insect which attack almost any part of host plan (*Aloe vera*) and causes damages to the most internal components of plant leaves.

Table 1. Effect of infestation by *I. aegyptiaca* on the internal components of *A. vera* during season 2018.

Plant	Part used	Internal components	Concentration mg/g	
			Non infested	Infested
Aloe vera	Leaves	Vitamin A	2.75^{c}	1.98^{a}
		Vitamin C	3.25^{b}	2.17^{a}
		Vitamin E	1.75^{b}	1.15^{c}
		Vitamin B12	2.35^{a}	1.57^{c}
		Folic acid	3.45^{c}	2.33^{b}
		Salicylic acids	1.76^{a}	1.76^{a}
		Amino acids	3.75^{c}	2.35^{a}
		Minerals	2.75^{b}	1.82^{a}
		Sugars	3.32^{a}	2.25^{c}
		Lignin	1.75^{a}	0.95^{c}
		Saponins	1.32^{a}	1.32^{a}
		Beta carotene	2.53^{b}	1.25^{c}
F (0.05)		234.85		
L.S.D		1.76		

2- Effect of *S. acupunctatus* on *A. Americana*:

Data tabulated in Table (2) show the effect of insect infestation by *S. acupunctatus* on the internal components of *A. americana*. Whereas concentration of most of Steroid glycosides such as Hecogenin, Clorogenin, Tigogenin and Smilagenin decreased from 2.15, 1.35, 1.15 and 1.76 mg/g to 1.23, 0.94, 0.75 and 0.95 mg/g, respectively. While concentration of Sisalagenin and Saponins did not change after infestation by *S. acupunctatus* .While concentration of total carbohydrates, total fat, total protein, total vitamins and total menerals decreased from 32.53, 4.52,1.63, 4.82 and 9.75 mg/g to 24.33, 3.45, 0.96, 3.54 and 7.66 mg/g, respectively.

Table 2. Effect of infestation by *S. acupunctatus* on the internal components of *A. Americana* during season 2018.

Plant	Part used	Internal components	Concentration mg/g	
			Non infested	Infested
Agave americana	Leaves	Steroid glycosides :		
		Hecogenin	2.15^{b}	1.23^{a}
		Clorogenin	1.35^{b}	0.94^{c}
		Tigogenin	1.15^{a}	0.75^{c}
		Sisalagenin	1.65^{b}	1.65^{b}
		Smilagenin	1.76^{c}	0.95^{a}
		Saponins	1.25^{a}	1.25^{a}
		Total carbohydrates	32.53^{b}	24.33^{c}
		Total fat	4.52^{a}	3.45^{c}
		Total protein	1.63^{b}	0.96^{c}
		Total vitamins	4.82^{a}	3.54^{c}
		Total menerals	9.75^{a}	7.66^{b}
F (0.05)		321.92		
L.S.D		1.88		

Statically analysis showed that were highly significant differences between concentrations of the most internal components of *A. americana* leaves which infested by *S. acupunctatus* compared to concentrations of the same components of non infested plants (control). Whereas F (0.05) value and L.S.D value were 321.92 and 1.88, respectively.

These results were agreement with those obtained by Cesar, R. *et al.* (2008) who reported that the agave weevil, *Scyphophorus acupunctatus* (Coleoptera : Curculionidae), is the most important insect pest of wild and cultivated agaves in the world. Pedro, F. and Fernando, S. (2013) reported that the agave weevil, *Scyphophorus acupunctatus* is the main pest of *Agave Americana* in Mexico

3- Effect of *A. nerii* on C. *procera*:

Data tabulated in Table (3) show the effect of insect infestation by *A. nerii* on the internal components of *C. procera*. Whereas concentration of Cardinolide glycosides, Trypsin, Alpha calotropeol and Beta calotropeol which found in the latex decreased from 1.98, 3.75, 5.86 and 4.65 mg/g to 0.75, 2.14, 3.95 and 3.52 mg/g, respectively. While concentration of Proteoclastic enzyme and Caoutchouc did not change after infestation by *A. nerii*. While concentration of Cardinolide glycosides, Usharin, Ushardin, Calotoxin and Calctin which found in leaves and flowers decreased from 1.85, 6.73,7.23, 5.64 and 3.75 mg/g to 0.75, 4.85, 5.75, 3.75 and 2.15 mg/g, respectively.

Table 3. Effect of infestation by *A. nerii* on the internal components of *C. procera* during season 2018

Plant	Part used	Internal components	Concentration mg/g	
			Non infested	Infested
Calotropis procera	Latex	Cardinolide glycosides	1.98^{c}	0.75^{a}
		Trypsin	3.75^{b}	2.14^{c}
		Alpha calotropeol	5.86^{a}	3.95^{b}
		Beta calotropeol	4.65^{c}	3.52^{a}
		Proteoclastic enzyme	2.75^{a}	2.75^{a}
		Caoutchouc	3.52^{a}	3.52^{a}
	Leaves @Flowers	Cardinolide glycosides	1.85^{a}	0.75^{c}
		Usharin	6.73^{c}	4.85^{b}
		Ushardin	7.93^{b}	5.75^{c}
		Calotoxin	5.64^{c}	3.75^{a}
		Calctin	3.75^{a}	2.15^{b}
		Calotropagenin	2.25^{b}	2.25^{b}
	Bark	Giganteol	7.75^{a}	5.95^{b}
		Iso giganteol	6.85^{a}	4.97^{c}
F (0.05)	272.59			
L.S.D	1.53			

While concentration of Calotropagenin did not change after infestation by *A. nerii*. Lastly, concentration of Giganteol and Iso giganteol which found in the bark decreased from 7.75 and 6.85 mg/g to 5.95 and 4.97 mg/g, respectively after infestation by *A. nerii*

Statically analysis showed that were highly significant differences between concentrations of the most internal components of *C. procera* which infested by *A. nerii* compared to concentrations of the same components of non infested plants (control). Whereas F (0.05) value and L.S.D value were 272.59 and 1.53, respectively

These results were agreement with those obtained by Al-Robai, A. (1997) and Al-Robai, A. et al. (1998) in Saudi Arabia who referred to the latex of various parts of

the Ushar milkweed , *Calotropis procera* and reported that the total of cardiac glycosides content of the latex changed.

According to the season and insects specially *A. nerii* . It was lowest in winter- spring when large numbers of aphid feed on the plant, and greatest in summer when only a few insects of aphid fed on the plant. Khan, *et al.* (1981) referred to the proteinase from the wild latex plant *C. procera* was extracted from leaves and stems and studied the effect of infestation by *A. nerii* on the internal components of this latex. El-Kady *et al*.(1980) in Egypt, reported that the stylets of *A. nerii* penetrated the epidermal layers intracellular but the ground tissue, the xylem and phloem tissues of the leaves and stems both intra and intercellular.

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أثر الإصابة الحشرية على بعض المكونات الداخلية لبعض أنواع نباتات الصبارات الهامة

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أجريت هذه الدراسة بغرض دراسة أثر الإصابة الحشرية على بعض المكونات الداخلية لبعض أنواع الصبارات الهامة. لذلك فإن هذه الدراسة شملت دراسة أثر الإصابة الحشرية لثلاثة حشرات هامة على المكونات الداخلية لثلاثة أنواع هامة من نباتات الصبار . حشرة البق الدقيقى المصرى (Douglas) *Icerya aegyptiaca*على صبار *Aloe vera* و حشرة سوسة الأجاف *Scyphophorus acupunctatus* على صبار *Agave americana* وحشرة من الدفلة *Aphis nerii* على صبار العشار *Calotropis procera* أجريت هذه الدراسة فى موقعين مختلفين هما حديقة الأورمان (محافظة الجيزة) و حديقة أنطونيادس (محافظة الإسكندرية) خلال عام 2018 . توصلت النتائج المتحصل عليها من تحليل المكونات الداخلية للنباتات المصابة بالحشرات موضع الدراسة ومقارنتها بالمحتويات الداخلية للنباتات السليمة الخالية من الإصابة الحشرية إلى تأثر معظم المحتويات الداخلية للنباتات المصابة بالحشرات موضع الدراسة وإنخفاض مستوى تركيزها بالمقارنة بالنباتات السليمة . هذا مع بقاء تركيزبعض المحتويات الداخلية كما هو لم يتغير بعد الإصابة الحشرية . ويتضح ذلك جليا من التحليل الإحصائى الذى يتمثل فى وجود فروق معنوية عالية بين تركيز تلك المحتويات الداخلية للنباتات المصابة بالحشرات المختلفة وتركيز تلك المحتويات فى النباتات السليمة الخالية من الإصابة الحشرية.