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Effectiveness of Different Infestation Levels by *Aphis nerii* on the Internal Components of Usher Milkweed, *Calotropis procera* cactus

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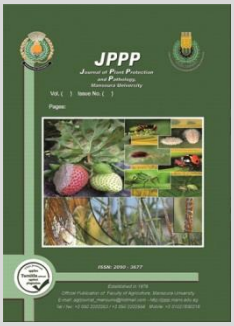

ABSTRACT

Experiments were carried out to evaluation effectiveness of different infestation levels by oleander aphid, *Aphis nerii* (Boyer) (Aphididae:Hemiptera) on quantity and quality of the internal components of important cactus-plant, Usher Milkweed *Calotropis procera* (Ait) (Fam:Asclepiadaceae). Experiments were carried out at two different locations; Giza and Alexandria Governorates during season 2022. Infestations by *A. nerii* during successive seasons were classified into three levels: low, medium and high (due-to mean population number of aphid). Internal components of cactus leaves were: cardiac-glycosides, usharin, ushardin, calotoxin, calctin, calotropagenin, trypsin, proteoclastic-enzyme alpha-calotropeol, beta-calotropeol, giganteol and iso-giganteol. Data obtained in both of two locations showed that the low infestation by *A. nerii* on *C. procera* affected on concentrations of the internal components of *C. procera* leaves compared-to control (healthy leaves) and medium infestation treatments had higher effect than low-infestation and control treatment. Further, high-infestation had greater effect than both of low and medium infestations. Quantity of usher Milkweed (latex) decreased in cactus-trees infested by *A. nerii* compared-to control in this descending order; high, medium and low infestation at both of twolocations. Concentrations (i.e. quality) of internal components of *C. procera* leaves significantly varied among levels of infestations including those of free-infestation level and quantity of usher Milkweed (latex) significantly differed as well.

Keywords: Oleander aphid *Aphis nerii*, cactus-plant, Usher Milkweed, *Calotropis procera*

INTRODUCTION

The Usher Milkweed Cactus, *Calotropis procera* (Ait) (Fam. Asclepiadaceae) commonly known as Aak or Madar plant is a perennial wild herb which plays various important ecological roles, Chandra *et al.* (2011). Also, *C. procera* cosider one of most important cactus plants which has many uses in medicinal purposes beside its essential uses in the decoration due to its beautiful flowers, Kumar *et al*. 2001. Also, Al-Robai *et al*. (1998) in Saudi Arabia mentioned to the important role of latex which extracted from various parts of the Usher Milkweed *C. procera* and indicated that latex contains many important total cardiac glycosides and they also mentioned that cardiac glycosides are varied according to the time of the day and season since, it was greatest in winter-spring and lowest in summer. De and Datt (1988) in India indicated that Usher Milkweed Cactus, *C. procera* has many important medicinal uses whereas its latex contain some important glycosides (cardiac glycosides) which used to treatment some heart diseases, cardiovascular and adjust blood glucose, and also contains many important component such as; usharin, ushardin, calotropagenin, calctin and calotoxin. Further, Khan *et al.* (1981) indicated that the important proteinase could be extracted from the leaves and flowers of wild latex plant *C. procera*.

The oleander aphid, *Aphis nerii* (Boyer) (Hemiptera: Aphididae) is one of the most important aphids infested field, greenhouse many ornamental plants worldwide, Mohammad *et al.* (2012); Serafina *et al.* (2018) penetrates the epidermal layers intracellular but the ground tissue, the xylem and phloem tissues of the leaves and stems both intra and intercellular.

The current study was carried out to evaluation effectiveness of different infestation levels by *A. nerii* on the quantity and quality of the internal components of Usher Milkweed *C. procera*

MATERIALS AND METHODS

Field design:

Experiments were conducted on Usher Milkweed Cactus, *Calotropis procera* (Ait) at two different locations (Governorates) El-Orman Garedn (Giza Governorate) and Antoniadis Garden (Alexandria Governorate) during season 2022. (Twelve) trees of cactus plant (*C. procera*) were tested at both of the two locations. These cactus trees were located in isolated area in both of the two successive gardens and divided into 4-four separated groups (repliecates) each one consists of three cactus trees. Three repliecates were artificially infested with *A. nerii* and the fourth one was left free of infestation as control. Each replect isolated from the other by special plastic wire (polyethylene) with very narrow halls (0.5mm). All recommended agricultural processes except the chemical treatment were conducted on the cactus trees both of the two locations. It is proven accurate observations of the infestation by the successive insect, *A. nerii* (adults and nymphs) and directly counting was conducted in random samples of the cactus trees (five leaves from each cactus tree from different sides) weekly at both of the two successive locations. Population fluctuation of *A. nerii* was monitored weekly during the period from beginning of February until end of June during season 2022.

Laboratory design:

Cactus leaves (*C. procera*) were collected from all examined repliecates and infestation by *A. nerii* was classified

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into three levels; low, medium and high infestation chemical composition of concentrations the internal components of cactus leaves; cardiac glycosides, usharin, ushardin, calotoxin, calctin, calotropagenin, trypsin, proteoclastic enzyme alpha calotropeol, beta calotropeol, giganteol and iso giganteol was determined at the plant physiology laboratory, Faculty of Science, Ain Shams University . These internal components were determined both in the three levels of the infestation and also in the control. The supernatant was used for gel analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970).

Statistical analysis:

Mean population number of *A. nerii* (adults and nymphs) on cactus plants *C. procera*, concentrations of the internal components of cactus leaves and quantity of usher Milkweed (latex) in cactus leaves were analyzed using ANOVA and the mean values compared with the least significant differences (L.S.D). Analysis were performed using SAS program (SAS Institute 1988).

RESULTS AND DISCUSSION

Experiments were carried out to evaluate effect of different infestation levels by the oleander aphid, *A. nerii* on quantity and quality of the internal components of the important cactus plant Usher Milkweed *Calotropis procera* (Ait).

Population fluctuation of *A. nerii* on *C. procera*

Results obtained and tabulated in Table (1) and Fig. (1) show population fluctuation of *A. nerii* (adults and nymphs) infesting *C. procera* cactus (leaves) at both of the two successive locations (Giza and Alexandria governorates) during season 2022.

Table 1. Population fluctuations of *A. nerii* on *C. procera* leaves at Giza and Alexandria Governorates during season, 2022

Date	Giza Governorate			Alexandria Governorate		
	Low	Medium	High	Low	Medium	High
1/2/2022	16.5	34.9	70.5	19.7	42.3	75.5
8/2/2022	17.2	35.9	72.7	20.5	45.7	77.9
15/2/2022	18.7	37.7	74.5	22.3	47.3	80.5
22/2/2022	19.8	39.5	76.8	24.5	49.5	83.7
1/3/2022	21.3	41.8	80.5	26.3	51.1	86.5
8/3/2022	22.2	43.7	81.9	28.5	53.8	90.2
15/3/2022	21.3	45.8	85.5	30.4	55.2	92.0
22/3/2022	22.0	47.3	88.2	33.5	58.4	95.5
29/3/2022	24.1	50.7	90.0	35.6	61.5	97.7
5/4/2022	25.0	54.2	92.5	36.0	65.1	99.5
12/4/2022	27.9	57.8	87.3	38.3	69.4	101.1
19/4/2022	29.5	59.5	88.0	39.0	73.3	103.5
26/4/2022	31.3	61.7	90.4	40.1	77.8	105.7
3/5/2022	33.5	63.8	93.6	42.7	79.3	107.0
10/5/2022	35.7	67.6	95.2	45.5	80.5	110.3
17/5/2022	36.5	70.5	97.1	42.3	77.6	105.3
24/5/2022	33.0	68.3	93.0	40.7	75.1	95.2
31/5/2022	32.5	65.7	90.1	38.5	70.1	87.5
7/6/2022	25.7	62.1	87.3	38.4	67.3	80.4
14/6/2022	24.9	60.5	83.5	35.4	62.2	77.7
21/6/2022	23.2	57.3	80.5	32.5	57.2	72.2
28/6/2022	21.4	55.1	75.3	30.7	55.3	70.5
Total	563.2	1181.4	1874.4	741.4	1375.0	1995.4
Mean	25.6	53.7	85.2	33.7	62.5	90.7
F(0.05)		346.38			421.66	
L.S.D		1,065			1.74	

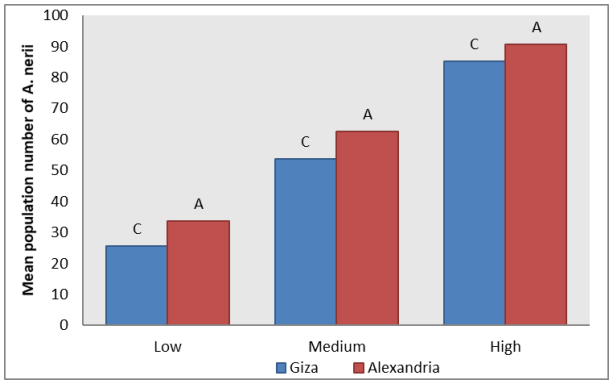

Fig. 1. Mean population of *A. nerii* on *C. procera* leaves at Giza and Alexandria Governorates during season 2022

In the end of season the infestation by *A. nerii* were classified into three levels of the infestation (low, medium and high) comparing to the mean population number of the successive insect *A. nerii* . Results obtained show that in Cairo Governorate the mean population number of *A. nerii* in low infestation was 25.6 individual/leaf, in medium infestation was 53.7 individual/leaf and in high infestation was 85.2 Individual/leaf. While in Alexandria Governorate the mean population number of *A. nerii* in low infestation was 33.7 individual/leaf, in medium infestation was 62.5 individual/leaf and in high infestation was 90.7 individual/leaf.

Statistical analysis show there were highly significantly differences between the mean population of *A. nerii* in the three levels of the infestation in both of the two locations

The obtained results are in agreement with those obtained by Behura and Bohidar (1983) in India who studied the effect of temperature on the fecundity of *A. nerii* and found that aphid died at 35 c and fecundity was highest at 30c and lowest at 15c. Also, Caralyn and Mark (2007) studied population dynamics of *A. nerii* and indicated to that the highest number of aphid found during the period from March to May. Also, Kataria and Kumar (2018) studied occurrence and infestation level of sucking pests (aphids) on various host plants and indicated to that The oleander aphid, *A. nerii* infested Usher Milkweed Cactus, *C. procera* during all year and has a high population number during period from March to May. Richard and Ehler (1980) studied population of *A. nerii* on oleander and indicated that the highest population of that aphid was found during the period from February to May. Also, Jarjes *et al.* (1989) in Iraq studied some ecological and biological aspects of *A. nerii* on some ornamental plants and found that there were significant positive and negative correlations between population density of the insect and temperature and relative humidity, respectively.

Effectiveness of insect infestation by *A. nerii* on the internal components *C. procera* Results in Table (2) show effect of the insect infestation by *A. nerii* on the internal components of cactus leaves at both of the two locations. The internal components were; cardiac glycosides, usharin, ushardin, calotoxin, calctin, calotropagenin, trypsin, proteoclastic enzyme, alpha calotropeol, beta calotropeol, giganteol and iso giganteol. The obtained data in both of the two locations show that the low infestation by *A. nerii* affected on concentrations (quality) of the internal components of *C.*

procera leaves whereas concentration of these components were declined after the infestation compared to control treatment and the medium infestation had a greater effect on concentrations than the low and zero infestation.The high infestation level had a greater effect on concentrations of the internal components than both of the low and medium infestations.

Statistical analysis revealed that there were highly significantly differences between concentrations of the internal components of *C. procera* leaves between the three levels of infestation in both of the two locations.

Table 2. Concentrations of the internal components of cactus plant *C. procera* (mg/ 100gm) at both of the three levels of the infestation by *A. nerii*

Component	Giza Governorate				Alexandria Governorate			
	Control	Low	Medium	High	Control	Low	Medium	High
Cardiac glycosides	5.7 [a]	5.0 [b]	4.2 [c]	3.5 [d]	6.2 [a]	5.7 [b]	5.0 [c]	4.8 [d]
Usharin	7.3 [a]	6.8 [b]	6.0 [c]	5.7 [d]	7.8 [a]	7.0 [b]	6.3 [c]	5.9 [d]
Ushardin	6.5 [a]	6.0 [b]	5.3 [c]	5.0 [d]	6.8 [a]	6.1 [b]	5.5 [c]	5.1 [d]
Calotoxin	8.7 [a]	8.1 [b]	7.8 [c]	7.3 [d]	8.4 [a]	8.0 [b]	7.5 [c]	7.2 [d]
Calctin	9.5 [a]	9.0 [b]	8.7 [c]	8.2 [d]	9.2 [a]	8.7 [b]	8.3 [c]	8.0 [d]
Calotropagenin	6.7 [a]	6.0 [b]	5.3 [c]	5.0 [d]	6.4 [a]	5.9 [b]	5.2 [c]	4.9 [d]
Trypsin	7.2 [a]	6.5 [b]	6.0 [c]	5.8 [d]	7.4 [a]	6.8 [b]	6.2 [c]	6.0 [d]
Proteoclastic enzyme	5.2 [a]	4.5 [b]	4.1 [c]	3.8 [d]	5.6 [a]	5.0 [b]	4.8 [c]	4.3 [d]
Alpha calotropeol	4.8 [a]	4.2 [b]	3.8 [c]	3.2 [d]	4.5 [a]	4.0 [b]	3.1 [c]	2.8 [d]
Beta calotropeol	3.9 [a]	3.2 [b]	2.7 [c]	2.5 [d]	3.6 [a]	3.3 [b]	3.0 [c]	2.5 [d]
Giganteol	4.2 [a]	3.9 [b]	3.5 [c]	3.0 [d]	4.0 [a]	3.3 [b]	2.9 [c]	2.6 [d]
Iso giganteol	3.9 [a]	3.4 [b]	3.0 [c]	2.7 [d]	3.7 [a]	3.2 [b]	2.8 [c]	2.3 [d]
F(0.05)	365.21				412.35			
L.S.D	1.002				1.003			

Effect of insect infestation by *A. nerii* on the quantity of usher milkweed (latex) of *C. procera* leaves

Results in Table (3) and Figure (2) show effect of the insect infestation levels by *A. nerii* on the quantity of usher milkweed (latex) of *C. procera* at both of the two locations.

Table 3. Quantity of the usher milkweed (latex) of cactus plant *C. procera* (mg/100gm) at Giza and Alexandria governorates

	Control	Low	Medium	High
Giza	25.3 [a]	20.5 [b]	18.2 [c]	15.5 [d]
Decreasing %	-	19.0	28.1	38.7
F(0.05)	311.27			
L.S.D	1.005			
Alexandria	27.4 [a]	21.8 [b]	20.0 [c]	17.3 [d]
Decreasing %	-	20.4	27.1	36.9
F(0.05)	425.33			
L.S.D	1.004			

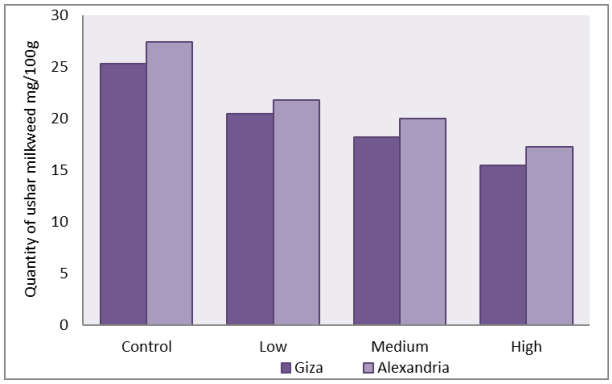

Fig. 2. Quantity of the usher milkweed (latex) (mg/100gm) of cactus plant *C. procera* leaves at Giza and Alexandria governorates

At Giza Governorate quantity of infested usher milkweed (latex) was 20.5, 18.2 and 15.5 mg/100g in the low, medium and high levels of infestation by *A. nerii* compared to control whereas it was 25.3 mg/100g and also the reduction percentage % of the quantity of usher milkweed (latex) at C. procera trees at both of the three levels of the infestations were arranged ascending as follow; 19.0, 28.1 and 38.7% while at Alexandria Governorate quantity of usher milkweed (latex) at C. procera trees infested by A. nerii at both of the three levels of the infestations; low, medium and high were arranged descending as follow; 21.8, 20.0 and 17.3 mg/100g compared to control whereas it was 27.4 mg/100g and also the reduction percentage % of the quantity of usher milkweed (latex) at C. procera trees at both of the three levels of the infestations were arranged ascending as follow; 20.4, 27.1 and 36.9%

Statistical analysis show revealed that there were highly significantly differences in quantity of usher milkweed (latex) between the three levels of infestation.

The obtained results are in agreement with those obtained by Marcio *et al.* (2022) who studied structural analysis revealed the interaction of cardenolides from Calotropis procera and indicated to that Aphis nerii feed on *C. procera* leaves and affected on about 35 chemically components extracted from this wild plant. Also, Dhafer *et al.* (2012) in The Kingdom of Saudi Arabia studied insects associated with milkweed, *C. procera* (Ait.) and indicated to that insect infestation by this insect affected on many important internal components of that wild cactus. Poomalai and David (1997) indicated that the insect infestation by yellow milkweed aphid, *A. nerri* Boyr on the wild cactus, *C. procera* affected on that plant phytochemistry such as protein, carbohydrate, lipids and cardenolides. Sastry *et al.* (2020) studied effect of insect infestation by aphid on two species of calotropis, Calotropis gigantean and Calotropis procera and indicated to that aphid, *A. nerii* affected on the internal components of cactus leaves. Emam (2002) in Egypt studied effect of some insects infestation on some ornamental plants and found that the insect infestation by *A. nerii* had significant effect on the cardiac glycoside in *C. procera*

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تأثير مستويات الإصابة المختلفة بحشرة *Aphis nerii* على المكونات الداخلية لنبات صبار العشار *Calotropis procera*

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الملخص

أجريت التجارب بغرض دراسة تأثير إصابة صبار العشار *Calotropis procera* بمستويات مختلفة من الإصابة الحشرية بحشرة من الدفلة *Aphis nerii* على المكونات الداخلية لصبار العشار. وقد أجريت التجارب فى منطقتين مختلفتين ; محافظة الجيزة ومحافظة الإسكندرية خلال عام 2022 ، وكانت المكونات الداخلية لصبار العشار التى تم تقديرها Cardiac-glycosides, Usharin, Ushardin, Calotoxin, Calctin, Calotropagenin, Trypsin, Proteoclastic-enzyme-alpha-calotropeol, beta-: calotropeol, giganteol and iso-giganteol. وقد أوضحت النتائج المتحصل عليها فى محافظة الجيزة إلى وجود إختلاف فى تركيز المواد الداخلية لصبار العشار فى حالة الإصابة بمستوى منخفض من الإصابة الحشرية بحشرة من الدفلة *A. nerii* وذلك بالمقارنة بنباتات العشار الغير مصابة بالحشرة (الكنترول) حيث قل تركيز تلك المواد فى النباتات المصابة بالحشرة مقارنة بالنباتات الغير مصابة (الكنترول). كما حدث وجود إختلاف أكثر فى تركيز هذه المواد الداخلية فى حالة الإصابة المتوسطة بالحشرة محل الدراسة *A. nerii* حيث كان تأثير الإصابة المتوسطة بالحشرة أكبر من تأثير الإصابة المنخفضة وذلك أيضا بالمقارنة بالنباتات الغير مصابة (الكنترول) . كذلك فإن تأثير الإصابة المرتفعة بالحشرة محل الدراسة كان أعلى من كلا من تأثير الإصابة المنخفضة و المتوسطة مقارنة بالكنترول. وعلى نفس المنوال كانت النتائج فى محافظة الإسكندرية. كما أشارت النتائج المتحصل عليها كذلك إلى تأثر كمية المادة اللبنية (لاتكس) التى تحتويها أوراق نباتات صبار العشار والتى تحتوى تلك المواد الفعالة حيث قلت نسبة تلك المادة اللبنية فى نباتات الصبار المصابة بحشرات المن *A. nerii* كما أشارت النتائج المتحصل عليها كذلك إلى أن إنخفاض نسبة تلك المادة اللبنية يزداد بازدياد مستوى الإصابة بالحشرة محل الدراسة وذلك فى موضعى الدراسة.