

Interactions Between *Aphis gossypii* (Glov.) And the Common Predators in Eggplant and Squash Fields, with Evaluating the Physiological and Biochemical Aspects of Biotic Stress Induced by Two Different Aphid Species, Infesting Squash and Cabbage Plants

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Abstract: This work was conducted to survey the major piercing sucking insect pests in eggplant and squash fields and their common associated predators, during summer plantations of seasons 2006 and 2007. The main physiological and biochemical effects that resulted from the attack of two different aphid species were also estimated. Stressed squash and cabbage plants mostly showed significant reductions in the contents of their metabolites including, chlorophylls, soluble sugars, total nitrogen, a total protein, total free amino acids and IAA, they also adapted an array of mechanisms to overcome the biotic stress, these mechanisms included, carotenoids, amino acids, free proline, ABA and stress induced proteins.

Key words: Vegetables, Eggplant, Squash, cabbage, Pests, Aphids, Predators, Amino acids, Hormones, Proteins.

INTRODUCTION

The importance of cultivating vegetables has extensively increased year after year; due to their economic value. However, most of these agricultural crops are subjected to attack by many piercing sucking insect pests such as aphids, whiteflies and jassides (El-Khawas, 2005). The cotton aphid (*Aphis gossypii* Glov.) is one of the most widespread species of aphids, and displays a large range of host-plants, covering very different families. This impressive behavior made it a major pest of numerous crops (Fuchsberg *et al.*, 2007). Also, many natural enemies (such as predators) play a noticeable role against different aphid species. Now, it is important to recognize both the positive and negative interactions that occur between the two primary components of a management system; plant cultivars and natural enemies, when developing Integrated Pest Management systems (El-Heneidy and Abdel-Samad, 2001).

As, induced multiple defense responses are elicited, when plants are exposed to biotic stresses such as attack by herbivores or pathogens (Sarosh and Meijer, 2007). Higher plants have intricate mechanisms enabling them to respond to environmental changes, most likely established over a long period of evolution as sessile organisms (Wu *et al.*, 2007). These plant responses are controlled at the molecular level by changes in gene expression and many genes are involved in such stress responses (Tardif *et al.*, 2007). Phytohormones may participate in stress perception signaling and possibly initiate a cascade of stress –induced responses (Chandler and Robertson, 1994). The current study was conducted for two goals; the first was to survey the major piercing sucking insect pests especially the cotton aphid *A. gossypii* and the common predators as biological agents, during summer plantations of eggplant and squash in the two successive seasons, 2006 and 2007, in Sharkia Governorate. While, the second one was to explore the effects of aphid herbivory of *A. gossypii* on squash plants and *Brevicoryne brassicae* on cabbage plants (*Brassica oleracea* var. *capitata*) (of the summer plantation of season, 2007), then deducing some physiological and biochemical mechanisms adapted by these plants to overcome such biotic stress caused by the two previous different aphid species.

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MATERIALS AND METHODS

Field studies:

The study was carried out in eggplant and squash fields, in Mashtol El-Souk district (Sharkia Governorate, Egypt), during the two successive seasons, 2006 and 2007. Summer plantations were selected for the survey purpose. Two adjacent areas of each one fadden (for each of the two studied crops) was chosen for recording the major piercing sucking insect pests attacking the two previous plants. These areas received all the normal agricultural practices except the use of chemical insecticides. Weekly samples of 200 leaflets (50 plants x4 leaflets/plant), for eggplant plantation and 200 leaves (50 plants x4 leaves/plant), for squash plantation, were examined at random. Examination started when the plants of each crop were 14 days old, in the first and second weeks of April 2006 and 2007, for eggplant and the third week of March 2006 and 2007, for squash. While, the survey ended in the second week of June and the last week of September 2006 and 2007, for eggplant and squash, respectively. Direct counts in the two fields of aphids (adults and nymphs), *Bemisia tabaci* (adults) and *Empoasca decipiens* (adults and nymphs), were carefully done early in the morning on both surfaces of either leaflets or leaves of the two studied plants. Also, the most common predators surveyed associated with such insect pests were counted directly in the fields. Then, the investigated samples were transferred in paper bags to the laboratory for further examination and actual counts in the same days of *B. tabaci* nymphs by the aid of a stereomicroscope.

Physiological and biochemical studies:

Fresh leaves of healthy and infested squash and cabbage of 45 days age of the summer plantation of season, 2007, were used for such studies. These samples were obtained from the same place and under the same conditions of those of the field studies.

Estimation of pigments:

Chlorophyll a, b and carotenoids contents were determined according to Saric *et al.* (1976).

Estimation of sugars:

Total soluble sugars were extracted following the method adopted by Homme *et al.* (1992) and determined with the anthrone reagent (Whistler *et al.*, 1962).

Estimation of proline:

Free proline was determined according to Troll (1995).

Protein analysis:

Soluble proteins were determined according to the method described by Bradford (1976). Electrophoretic protein profile was analyzed by SDS-PAGE technique (Laemmli, 1970). Data were analyzed and identified by gel documentation system (GDS) with standard markers using Gel Proanalyzer Version 3 Media Cybernetics Tice Imaging Experts Software.

Estimation of indol acetic acid (IAA) and abscisic acid (ABA):

They were extracted and separated following the method adopted by Shindy and Smith (1975). Identification and quantification were carried by GLC (Varien Vesta, 6000) using the method described by Vogel (1975).

Total free amino acids:

They were determined by the method described by Jayaraman (1985) and were analyzed with HPLC Amino acid analyzer LC 3000 eppendorf (Germany) according to the instruction's manual (1999).

Statistical analysis:

The obtained data were statistically analyzed according to procedures outlined by Snedecor and Cochran (1980) and least significant differences (L.S.D.) test was run to compare the mean values at 1% and 5% level of probability using SAS program (SAS Institute, 1994). The means of temperature and relative humidity were obtained from the Meteorological station of Agricultural Research Center (A.R.C.).

RESULTS AND DISCUSSIONS

Field studies:**Eggplant summer plantation:**

The cotton aphid, *Aphis gossypii* (Glov.) (Homoptera: Aphididae) was the only recorded aphid species attacking eggplant during the summer plantations of the two seasons, 2006 and 2007. Similarly, Mousa (2003) recorded *A. gossypii* as a pest of eggplant. From Table (1), the aphid populations increased gradually until reaching their maximum total numbers during the last week of April, 2006 and 2007, respectively (631&483 individuals/200 leaflets). Then, they decreased until disappeared in the end of season (in the last week of September, 2006 and 2007).

Table 1: Total numbers of major piercing sucking insect pests (adults & nymphs), attacking summer plantations of eggplant, during seasons, 2006 and 2007, in Sharkia Governorate.

Season 2006							Season 2007						
Dates of sampling	1	2	3	Total no.	Weather factors		Dates of sampling	1	2	3	Total No.	Weather factors	
					Mean °C	Mean R.H.%						Mean °C	Mean R.H.%
8/4/2006	0	0	0	0	20.0	51.0	7/4/2007	0	0	0	0	23.3	47.0
15/4	99	0	0	99	19.0	54.0	14/4	30	3	0	33	18.9	49.0
22/4	198	7	1	206	22.5	55.0	21/4	181	7	1	189	19.5	48.0
29/4	631	15	2	648	24.0	60.0	28/4	483	12	0	495	20.8	49.0
6/5	317	45	7	369	28.0	54.0	5/5	214	18	1	233	22.0	50.0
13/5	296	60	18	374	24.2	58.0	12/5	193	27	3	223	23.1	50.0
20/5	281	83	30	394	21.2	55.0	19/5	167	43	7	217	29.0	48.0
27/5	193	143	34	370	28.9	53.0	26/5	145	70	11	226	27.6	48.0
3/6	151	159	43	353	30.5	58.0	2/6	130	134	14	278	23.5	49.0
10/6	130	185	63	378	22.4	63.0	9/6	122	158	37	317	26.3	49.0
17/6	126	287	95	508	27.7	60.0	16/6	110	219	91	420	25.9	50.0
24/6	114	162	41	317	32.3	62.0	23/6	114	127	106	347	27.7	49.0
1/7	113	74	14	201	32.3	60.0	30/6	112	66	119	297	29.1	49.0
8/7	121	78	19	218	30.5	62.0	7/7	36	87	121	244	27.8	50.0
15/7	116	170	28	314	31.3	63.0	14/7	49	116	129	294	26.5	50.0
22/7	120	283	30	433	30.2	63.0	21/7	68	195	138	401	29.2	50.0
29/7	141	508	41	690	30.6	65.0	28/7	72	321	155	548	30.2	51.0
5/8	167	672	98	937	30.6	67.0	4/8	86	486	168	740	27.0	50.0
12/8	147	1428	113	1688	30.0	65.0	11/8	125	749	172	1046	27.6	51.0
19/8	173	417	127	717	31.3	65.0	18/8	149	426	178	753	26.8	51.0
26/8	183	361	163	707	30.2	66.0	25/8	158	359	323	840	30.0	51.0
2/9	238	248	298	784	29.3	63.0	1/9	193	212	422	827	27.5	51.0
9/9	150	74	107	331	30.1	62.0	8/9	184	80	147	411	26.3	51.0
16/9	27	19	34	80	29.0	68.0	15/9	17	26	63	106	24.2	51.0
23/9	0	0	0	0	28.7	63.0	22/9	0	0	0	0	25.5	51.0
Mean/season	169.28	219.12	56.24	444.64	27.8	60.6	Mean/season	125.52	157.64	96.24	379.40	25.5	49.8
	A	A	B	(0 - 631)	(19.0 - 32.3)	(51.0 - 68.0)		B	B	A	(0 - 483)	(18.9 - 30.2)	(47.0 - 51.0)

Statistical analysis:

L.S.D. 0.05 (2 seasons)

A. gossypii
3.009

B. tabaci
3.848

E. decipiens
1.555

1- *A. gossypii* (adults & nymphs).

2- *B. tabaci* (adults & nymphs).

3- *E. decipiens* (adults & nymphs).

Note: Means of the same letter are not significantly different.

The most common observed predators found associated with the major piercing sucking insect pests were belong to 4 families including the following; *Chrysopa carnea* (Steph.) (Neuroptera: Chrysopidae), *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae), *Orius laevigatus* (Fiber.) (Hemiptera: Anthocoridae), *Scymnus* sp. (Coleoptera: Coccinellidae) and *Syrphus* sp. (Diptera: Syrphidae). True spiders (unidentified species) were also recorded during the study. The maximum total numbers of all surveyed predators (80 and 66 individuals /200 leaflets), were recorded during the third week of June, 2006 and 2007, respectively. Frempong and Buahin (1978) suggested from the regular occurrence of predators throughout the growing season, that they had an appreciable influence in the regulation of pest populations attacking eggplant. Besides, it was clear from Table (2) that ,the time of occurrence of predators were differed from each predator to another. Consequently,

Table 2: Total numbers of surveyed predators in eggplant fields, during summer plantations of seasons, 2006 and 2007, in Sharkia Governorate.

Season 2006							Season 2007								
Dates of sampling	Predators						Total no.	Dates of sampling	Predators						Total no.
	1	2	3	4	5	6			1	2	3	4	5	6	
8/4/2006	0	0	0	0	0	0	0	7/4/2007	0	0	0	0	0	0	0
15/4	0	0	0	0	0	0	0	14/4	0	0	0	0	0	0	0
22/4	0	1	0	0	0	0	1	21/4	0	2	0	0	0	0	2
29/4	0	2	0	1	0	1	4	28/4	0	1	0	0	0	1	2
6/5	1	4	0	3	0	1	9	5/5	0	16	0	2	0	2	20
13/5	3	7	0	7	2	3	22	12/5	1	12	0	4	3	4	24
20/5	9	11	0	12	4	4	40	19/5	2	15	0	7	8	6	38
27/5	18	8	0	16	3	3	48	26/5	7	8	0	13	5	3	40
3/6	33	6	0	19	1	3	62	2/6	13	4	0	19	3	1	44
10/6	45	2	0	25	0	4	76	9/6	24	1	0	23	1	2	54
17/6	54	0	0	20	0	6	80	16/6	46	0	0	16	0	3	66
24/6	50	0	0	16	0	8	74	23/6	35	0	0	9	0	2	46
1/7	38	0	0	11	0	4	53	30/6	28	0	0	4	0	1	33
8/7	25	0	0	6	0	7	38	7/7	25	0	0	3	0	2	30
15/7	16	0	0	3	0	7	26	14/7	21	0	0	1	1	5	28
22/7	14	0	0	2	0	4	20	21/7	17	0	0	1	1	8	27
29/7	12	0	0	0	0	10	22	28/7	12	0	0	0	0	12	24
5/8	9	0	1	0	0	9	19	4/8	10	0	0	1	0	6	17
12/8	6	0	1	0	0	11	18	11/8	9	0	0	1	0	4	14
19/8	5	0	1	1	0	9	16	18/8	7	0	1	0	0	2	10
26/8	4	0	2	2	0	7	15	25/8	4	0	2	0	0	2	8
2/9	3	0	5	1	0	5	14	1/9	2	0	4	0	0	1	7
9/9	2	0	7	1	0	2	12	8/9	0	0	5	0	0	1	6
16/9	1	0	2	0	0	1	4	15/9	0	0	1	0	0	1	2
23/9	0	0	0	0	0	0	0	22/9	0	0	0	0	0	0	0
Total	348	41	19	146	10	109	673	Total	263	71	13	104	22	69	542
/season	(0-	(0-	(0-	(0-	(0-	(0-	(0-	/season	(0-	(0-	(0-	(0-	(0-	(0-	(0-
(range)	54)	11)	7)	25)	4)	11)	80)	(range)	46)	15)	6)	23)	8)	12)	66)

1- *C. carnea* (adults & larvae).
 2- *C. undecimpunctata* (adults & larvae).
 3- *Orius* sp. (adults & nymphs).
 4- *Scymnus* sp. (adults & larvae).
 5- *Syrphus* sp. (larvae).
 6- True spiders.

their percentages of occurrence to each others were variable. The two predators; *C. carnea* and *Scymnus* sp., were the most abundant species among other all surveyed predators on eggplant. The ratio between the major piercing sucking insect pests and common predators on summer plantations of eggplant was shown in Table (5).

Squash summer plantation:

The cotton aphid *A. gossypii* was the only common recorded aphid species, attacking squash plantations. Results were confirmed by those obtained by Mohamed (1996). Aphid populations increased gradually until reaching their maximum total numbers (2839 & 4309 individuals/200 leaves), during the last week of April, 2006 and 2007, respectively (Table, 3). Our results were similar to those obtained by Banerjee *et al.* (1986) who found that, *A. gossypii* attacked cucurbit crops and its maximum population occurred during April.

Moreover, the predatory species observed in squash fields, were the same as those surveyed in eggplant fields. The highest total numbers of these predators were recorded during the second week of May, 2006 and 2007 (163 and 127 individuals/200 leaves, respectively). Pereira and Smith (1976) indicated that, Syrphidae, Coccinellidae and Chrysopidae were found preying on aphids infesting squash. The extended time of occurrence of predators were differed from each predator to another. Consequently, their percentages of occurrence to each others were variable. The three predators; *C. carnea*, true spiders and *C. undecimpunctata*, were the most abundant predatory species surveyed in squash fields. The ratio between the major piercing sucking insect pests and common predators on squash plants were recorded in Table (5).

In conclusion, field studies indicated that, the periods of occurrence of common predators observed in both eggplant and squash fields were synchronized with that of the major piercing sucking insect pests. Thus, indicating the important natural role of such biocontrol agents against these insect pests. Magnifying this role has become necessary for further releases of most of their common recorded species such as;

Table 3: Total numbers of major piercing sucking insect pests (adults & nymphs), attacking summer plantations of squash, during seasons, 2006 and 2007, in Sharkia Governorate.

Season 2006							Season 2007						
Dates of sampling	1	2	3	Total no.	Weather factors		Dates of sampling	1	2	3	Weather factors		Total no.
					Mean °C	Mean R.H.%					Mean °C	Mean R.H.%	
18/3/2006	0	0	0	0	20.3	58.0	17/3/2007	0	0	0	0	13.5	46.0
25/3	120	11	0	131	22.8	50.0	24/3	46	3	0	49	16.2	45.0
1/4	467	27	0	494	20.5	55.0	31/3	165	61	0	226	20.9	47.0
8/4	1695	82	1	1778	20.0	51.0	7/4	871	96	0	967	23.3	47.0
15/4	2747	103	5	2855	19.0	54.0	14/4	1356	111	1	1468	17.4	49.0
22/4	2768	583	9	3360	22.5	55.0	21/4	3678	311	2	3991	19.5	48.0
29/4	2839	177	15	3031	24.0	60.0	28/4	4309	159	8	4476	20.8	49.0
6/5	1160	144	23	1327	28.0	54.0	5/5	1825	147	11	1983	22.2	50.0
13/5	583	219	40	842	24.2	58.0	12/5	760	126	16	902	23.1	50.0
20/5	110	168	71	349	21.2	55.0	19/5	372	104	34	510	29.0	48.0
27/5	46	57	32	135	28.9	53.0	26/5	185	41	10	236	27.6	48.0
3/6	8	15	7	30	30.5	58.0	2/6	29	6	2	37	23.5	49.0
10/6	0	0	0	0	25.9	63.0	9/6	0	0	0	0	26.3	49.0
Mean	964.85	122.00	15.62	1102.47	23.7	55.7	Mean/season	1045.85	89.62	6.45	1141.92	21.8	48.1
(range)	B (0-2839)	A (0-583)	A (0-71)	(0-3360)	(19.0-30.5)	(50.0-63.0)	(range)	A (0-4309)	B (0-311)	B (0-34)	(0-4476)	(13.5-29.0)	(45.0-50.0)

Statistical analysis:

L.S.D. 0.05 (2 seasons)	<i>A. gossypii</i>	<i>B. tabaci</i>	<i>E. decipiens</i>
	29.733	3.171	0.361

1- *A. gossypii* (adults & nymphs). 3- *E. decipiens* (adults & nymphs).

2- *B. tabaci* (adults & nymphs).

Note: Means of the same letter are not significantly different.

Table 4: Total numbers of surveyed predators in squash fields, during summer plantations of seasons, 2006 and 2007, in Sharkia Governorate.

Season 2006							Season 2007								
Dates of sampling	Predators						Total no.	Dates of sampling	Predators						Total no.
	1	2	3	4	5	6			1	2	3	4	5	6	
18/3/2006	0	0	0	0	0	0	0	17/3/2007	0	0	0	0	0	0	0
25/3	0	1	2	2	0	0	5	24/3	0	2	1	0	0	1	4
1/4	0	2	0	2	0	0	4	31/3	1	2	0	0	0	1	4
8/4	0	3	0	3	0	1	7	7/4	3	16	5	1	0	5	30
15/4	0	10	0	5	0	2	17	14/4	17	24	1	3	1	6	52
22/4	1	41	1	13	0	6	62	21/4	28	56	1	11	2	11	109
29/4	9	32	3	10	1	10	65	28/4	36	37	1	10	2	24	110
6/5	28	34	6	9	1	36	114	5/5	42	26	1	8	3	46	126
13/5	75	15	10	3	1	59	163	12/5	46	8	4	6	5	58	127
20/5	32	6	17	2	2	25	84	19/5	15	6	2	4	1	28	56
27/5	17	1	5	1	0	7	31	26/5	7	2	1	3	0	10	23
3/6	6	0	2	0	0	3	11	2/6	3	0	0	1	0	3	7
10/6	0	0	0	0	0	1	1	9/6	1	0	0	0	0	1	2
Total/season	168	145	46	50	5	150	564	Total/season	199	179	19	47	14	194	650
(range)	(0-75)	(0-41)	(0-17)	(0-13)	(0-2)	(0-59)	(163)	(range)	(0-46)	(0-56)	(0-4)	(0-11)	(0-5)	(0-58)	(127)

1- *C. carnea* (adults & larvae).

2- *C. undecimpunctata* (adults & larvae).

3- *Orius* sp. (adults & nymphs). 6- True spires.

4- *Scymnus* sp. (adults & larvae).

5- *Syrphus* sp. (larvae).

C. carnea & *Scymnus* sp. in eggplant fields and *C. carnea* & *C. undecimpunctata* in squash fields and also other fields that are attacked by the major piercing sucking insect pests, especially *A. gossypii*. Using such biocontrol agents must be included and encouraged when planning I.P.M. strategies against them.

Physiological Studies:

In nature, plants are often subjected to biotic stress, where they elicited defense responses (Bruce and Pickett, 2007). However, aphids are destructive agricultural pests as they probe thin-walled sieve tubes and eject saliva into cells that they have probed, before commencing feeding. The saliva is thought to be

Table 5: Percentages of occurrence and ratios between major piercing sucking insect pests and their common associated predators, in eggplant and squash fields, during summer plantations of seasons, 2006 and 2007, in Sharkia Governorate.

Species	Season 2006		Season 2007		Mean percentages /2 seasons		
	Eggplant	Squash	Eggplant	Squash	Eggplant	Squash	
I. Pests	<i>A. gossypii</i>	38.07	87.52	33.08	91.58	35.78	89.59
	<i>B. tabaci</i>	49.28	11.07	41.55	7.85	45.72	9.43
	<i>E. decipiens</i>	12.65	1.41	25.37	0.57	18.50	0.98
	Total	100.00%		100.00%		100.00%	
II. Preda-tors	<i>C. carnea</i>	51.71	29.79	48.52	30.62	50.11	30.21
	<i>C. undecimpunctata</i>	6.09	25.71	13.10	27.54	9.59	26.63
	<i>Orius</i> sp.	2.82	8.16	2.40	2.62	2.61	5.39
	<i>Scymnus</i> sp.	21.69	8.87	19.19	7.23	20.44	8.05
	<i>Syrphus</i> sp.	1.49	0.88	4.06	2.15	2.78	1.51
	True spiders	16.20	26.59	12.73	29.84	14.47	28.21
	Total	100.00%		100.00%		100.00%	
Plant species/ months	Season 2006		Season 2007		Monthly ratio (2 seasons)		
Eggplant	April	190.60:1		179.70:1		185.56:1	
	May	12.66:1		7.37:1		9.98:1	
	June	5.33:1		6.83:1		6.01:1	
	July	11.67:1		13.64:1		12.47:1	
	August	59.54:1		68.96:1		63.49:1	
	September	39.83:1		89.60:1		56.42:1	
	Ratio/season	16.52:1		17.50:1		General ratio/(2 seasons)16.96:1	
Squash	March	26.20:1		34.38:1		31.23:1	
	April	74.31:1		36.22:1		49.17:1	
	May	6.77:1		10.94:1		8.68:1	
	June	2.50:1		4.11:1		3.19:1	
	Ratio/season	25.41:1		22.84:1		General ratio/(2 seasons) 24.03:1	

responsible for the onset of plant responses (Matsiliza and Botha, 2002). Additionally aphids do less structural damage, but are capable of themselves becoming 'sinks' to which all or most of the plant sap is diverted (Girousse *et al.*, 2003). Herbivory alter both the physiological and biochemical picture of the stressed plants (El-Khawas and El-khawas, 2005). Plants tend to be smaller due to loss of assimilates, or reduced leaf water potential (Cabrera *et al.*, 1994). Chlorosis reflects the reduction in chlorophyll content of infested plants (Wang *et al.*, 2004). Aphid infestations significantly reduce both chlorophyll a and b and consequently the total soluble sugars (as product of photosynthesis) in both plant leaves (Table, 6). However, the reduction was more significant in cabbage plant. Similarly, negative effects of aphids on plant photosynthesis have been reported in wheat (Macedo *et al.*, 2003). Gomez *et al.* (2006) reported that damage by aphids is due to consumption of phloem sap, and then the excretion of honeydew, which composed of large amounts of carbohydrates that are lost from aphid-infested plants and covers the leaves. Photosynthesis reduction may be due to unbalanced Chlorophyll biosynthesis (as result of alteration in mineral nutrition and degradation (as result of degradative enzymes) (Wang *et al.*, 2004). On the other hand, carotenoids showed a significant increase as a protective agent, secondary compounds, they play an essential role in resistance to aphids (Leszczynski *et al.*, 1989).

Table 6: Changes in chlorophyll (a & b) and total soluble sugars of squash and cabbage leaves in response to herbivory with *A. gossypii* and *B. brassicae* (the values listed were means of four samples).

Sample	Chlorophyll a (ug/g f.wt.)	Chlorophyll b (ug/g f.wt.)	Carotenoids (ug/g f.wt)	Total soluble sugar (mg/g f.wt)
Squash (control)	5.67 A	2.10 B	6.51 B	7.35 B
Squash (infested)	4.98 B	1.73 C	6.68 B	1.09 C
Cabbage (control)	5.83 A	2.62 A	2.20 C	7.95 A
Cabbage (infested)	1.20 C	0.68 D	8.65 A	1.23 C
L.S.D.0.01	0.246	0.098	0.347	0.303
L.S.D.0.05	0.169	0.067	0.239	0.208

Note: Values of the same letter are not significantly different.

Effect on Nitrogen Metabolism:

The total nitrogen and total free amino acid contents were mostly significantly reduced in both hosts as being compared to the controls, (Table, 7). The fractionation of the non protein amino acids using 15 authentic amino acids in addition to NH4 (Table, 8) revealed the separation of the same number of amino acids in both the control and infested plants, thus shows that the response to infestation is quantitative and not qualitative. In addition, most of the nitrogen content in squash is in the form of aspartic, while that in cabbage is glutamic

Table 7: Changes in total nitrogen, total soluble protein, total amino acids, proline, indole acetic acid and abscisic acid contents of squash and cabbage leaves in response to herbivory with *A. gossypii* and *B. brassicae* (values listed were means of four samples).

Sample	Total Nitrogen (mg/100 g d.wt.)	Total Protein (mg/100 g d.wt.)	Total free amino acids (mg/100 g d.wt.)	Proline (mg/100 g d.wt.)	Indole acetic acid (mg/100 g f.wt.)	Abscisic acid (mg/100 g f.wt.)
Squash (control)	220 A	190 A	24 A	5.5 D	16.0 A	3 D
Squash (infested)	170 C	130 B	16 C	9.1 D	5.8 C	14 B
Cabbage (control)	200 B	120 C	18 B	6.8 D	7.0 B	4 C
Cabbage (infested)	160 D	80 D	16 C	32.8 A	6.3 C	18 A
L.S.D.0.01	8.651	7.443	1.100	1.020	0.536	0.640
L.S.D.0.05	5.945	5.115	0.800	0.700	0.369	0.440

Note: Values of the same letter are not significantly different.

Table 8: Changes in free amino acids of leaves of squash and cabbage analyzed with HPLC Amino acid analyzer LC 3000.

Amino acids (mg/100 g d.wt.)	Squash		Cabbage	
	Control	Infested	Control	Infested
Aspartic acid	101.0	95.4	59.0	40.7
Theronine	10.1	9.8	21.5	13.0
Serine	15.0	15.5	25.6	23.4
Glutamic acid	64.3	46.4	110.3	90.5
Alanine	21.4	20.6	28.9	16.4
Cystein	50.0	43.9	78.7	57.2
valine	3.1	1.1	4.9	2.3
methionine	12.9	11.5	18.8	11.5
Isoleucine	197	17.2	32.2	14.8
Leucine	6.9	6.5	10.6	4.4
Tyrosine	18.5	14.5	25.7	17.0
Phenylalanine	2.8	0.7	4.1	2.5
Histidine	8.3	5.6	9.6	6.7
Lysine	15.9	11.6	24.1	12.6
NH ₄	64.3	39.3	40.8	58.3
Arginine	20.7	11.7	17.3	9.1

acids, in agreement with these results the major nitrogen carrier in a *Cucurbita maxima* phloem sap was reported to be aspartic acid (Richardson *et al.*, 1982). In general, there was an obvious reduction in the contents of the analyzed amino acids; with more severe reduction in cabbage plants. Thus, the fact that the pest is a sink of drain through consuming the amino acids of the plants is supported in this current work. Furthermore, there was a common reduction in 6 amino acids, which were aspartic acid, glutamic acid, cystein, tyrosine, phenylalanine and arginine. However, cystein may be conjugated into protein in the form of proteinase inhibitor (Pechan *et al.*, 2000). The reduction in methionine in cabbage may be directed towards the biosynthesis of ethylene or spermine. Also, arginine may be directed towards the synthesis of canavanine, an analog of arginine which has powerful antimetabolic effect on broad array of organisms. Canavanine is less basic than arginine. So, the polypeptide chain that contain canavanine instead of arginine needs less basicity that is required for protein folding and this may explain the accumulation of NH₄ (42.9% over the control) as a result of the severe reduction in arginine of infested cabbage (Orcut and Nilsen, 2000), this mechanism is obvious in cabbage. Moreover, infestation induced significant increases in the proline content, as an adaptive mechanism especially in cabbage (Table, 7). Proline is capable of movement between tissues, and is believed to protect plant against stress by acting as a storage compound for both carbon and nitrogen sources, thus protect cytoplasmic enzymes and cellular structure (Serrano and Gaxiola, 1994). The high sensitivity of the metabolic processes of proline synthesis and degradation themselves may be benefit by regulating metabolic processes which are adversely affected by stress (Hare and Cress, 1997).

IAA and ABA:

Signaling pathways involving the plant hormones which play a central role in integrating and coordinating whole plant stress responses (Smith and Boyko, 2007). The present investigation showed significant reduction in IAA contents of both infested plants (Table, 6). The reduction in IAA content against infestation may be due to the inhibition of biosynthesis of certain enzymes responsible for the indole compounds or directing the activity of these enzymes towards the formation of other compounds rather than the precursor tryptophan, or increasing the IAA oxidase activity (Kefeli, 1978). However, the reduction of amino acid phenylalanine, against

infestation is the logical explanation of the reduction in the biosynthesis of indole acetic acid (Table, 7). It is well known that, phenylalanine is directed towards the biosynthesis of tryptophane; which is the precursor of indole acetic acid, this mechanism is more obvious in squash.

Concerning ABA, their contents were increased in squash and cabbage infested plants (Table, 7), this increase may be an adaptive response and defense mechanism against aphid infestation (Hopkins and Hüner, 2004). The primary action of ABA may be an alteration of membrane properties such as membrane hyper-polarization. Thus, ABA is implicated in the control of physiological and molecular processes involved to face stress. In general, ABA induces gene activation that shows defensive protein in the cytoplasm (Falco *et al.*, 2001).

Proteins:

Proteins play a role in eliciting the response of resistant plants. It is clear from Table (9) and Fig. (1), that there are both qualitative and quantitative changes. The infested host leaves possessed low level of total soluble protein (Table, 7); this may be a subsequent result of the reduction of its biosynthesis or the drain of assimilates as amino acids by the aphid. The number of bands separated in both the controls is less than in both infested plants, thus *de novo* stress induced protein bands appeared in each as defense mechanism against herbivory (qualitative changes), in case of squash mid proteins appeared (66 and 54 kDa). On the other hand, cabbage induced proteins were high (101 kDa) and mid (54 and 48 kDa). The mid protein (54 kDa) is common in both hosts (Table, 9). There were 6 common bands in all bands are of low molecular weight (37, 35, 33, 30, 20 and 15 kDa), their intensities were more in infested plants and this is quantitative changes. The above results are in accordance with the suggestion of Azzouz *et al.* (2005), that the mechanism of aphid resistance may be explained by increased synthesis or expression of specific plant proteins, which enhance plant resistance to insect.

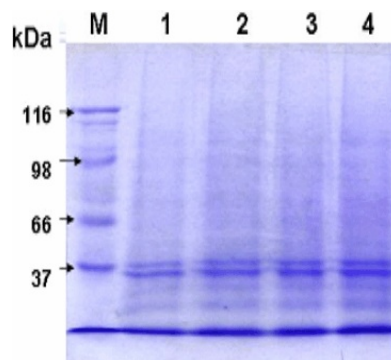


Fig. 1: Protein banding pattern of plant hosts. Lane 1: Protein marker, Lane 2: Protein of control squash, Lane 3: Protein of infested squash, Lane 4: Protein of control cabbage and Lane 5: Protein of infested cabbage.

Wounding results in the rapid formation and deposition of callose (Botha and Matsiliza, 2004). In addition, callose has an integral effect on the regulation of plasmodesmatal pore size (Botha and Cross, 2001), where it is deposited in the neck region of the plasmodesmata and may block them completely (Botha, 2005), altering the size exclusion limit of the plasmodesmata, thereby primarily reducing sap loss from the phloem. Callose is the major substance produced by plant cells in response to wounding (including aphid feeding). It follows then that, the resistant gene or gene system must affect callose production and deposition in some way. This could involve signals that elicit callose response, or expression of genes that produce the enzyme systems responsible for the production of callose itself. Cherqui *et al.* (2003) found that, B-glucuronidase (GUS) protein is responsible for the probiotic effects on aphid feeding, these enzymes are responsible for the breakdown of callose and may be an active defense against aphid salivary toxins, or may be against pathogens that may be introduced into the plant by the aphid saliva (Van der Westhuizen *et al.*, 2002). Furthermore, Pontoppidan *et al.* (2003) concluded that, the myrosinase-glucoinolate system present in crucifers is believed to serve as an important barrier to general herbivores, whereas specialist insect rely on this system for host recognition.

It might be concluded that, tolerance of squash and cabbage plants to aphid infestation includes: - (1) increasing in carotenoids as a protective agent, (2) reduction in amino acids as cystein which may be conjugated into protein in the form of proteinase inhibitor. The reduction in methionine in cabbage may be

Table 9: Changes in protein profiles and percentage of bands intensity of squash and cabbage leaves in response to herbivory against *A. gossypii* and *B. brassicae*.

Band no.	% of bands intensity of squash		% of bands and intensity of cabbage		M.Wt.
	Control	Infested	Control	Infested	
1	-	-	-	0.6	101
2	0.9	1.0	1.5	1.5	98
3	1.1	1.3	1.5	1.5	88
4	2.0	2.5	2.0	2.0	73
5	-	2.8	1.8	2.0	66
6	-	3.0	-	2.8	54
7	-	-	-	3.4	48
8	7.1	7.4	8.0	8.0	45
9	9.2	10.0	14	14.5	37
10	11.0	12.8	11.8	12.9	35
11	15.0	16.2	16	17	33
12	12.0	13.4	10	11	30
13	1.9	2.0	4.3	4.4	27
14	2.2	2.4	5.6	2.2	24
15	7.6	8.0	8.0	8.2	20
16	6.9	7.3	7.2	7.4	15
17	1.8	1.9	1.5	1.5	10
18	1.4	1.6	1.8	1.8	9
Total	14	16	15	18	

directed towards the biosynthesis of ethylene or spermine and reduction in argentine, which may be directed towards the synthesis of canavanine, an analog of arginine; (3) by elevating the free proline content; (4) increasing ABA contents; (5) two *de novo* stress induced protein bands in case of squash mid proteins appeared (66 and 54 kDa) and in cabbage induced proteins were high (101 kDa) and mid (54 and 48 kDa). The mid protein (54 kDa) is common in both.

At the end of our research, we suggest that producing genetically modified plant having either some or all of the previous defensive mechanisms will be benefit in coordination with the biological agents to perform an achievement in the field of IPM.

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