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Is there a Relationship between the Level of Plant Metabolites in Cucumber and Globe Cucumber and the Degree of Insect Infestation?

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Abstract

Cucumber plant was infested by three major insect pests, i.e., cotton aphid, white fly nymph and green bugs while globe cucumber, the wild cucumber species was kept healthy without any remarkable infestation. In addition, globe cucumber had higher ratios of T.S.S over T.N, T.S.S over T.C.A.A., T.S.S over T.F.A.A., higher content of cucurbitacins and phenolic compounds than cucumber in the two successive seasons. Lower values of these markers rendered cucumber susceptible to pests under study, not at all stages of growth but whenever these values were much lower through the growth stages. On the other hand, the major cucurbitacins were isolated and purified from each plant and identified by spectroscopic techniques. The development of cotton aphid treated with various globe cucumber extracts as well as the isolated cucurbitacins was also evaluated. Ethanol extract of globe cucumber caused higher mortality and affected on the biological aspects of cotton aphids when compared with all extracts. The importance of acetyl group and the double bond in the position 1 and 2 in different cucurbitacins on aphid's mortality was studied. A field study showed a remarkable reduction (82.0 %) of cotton aphid induced by spraying the cucumber with 4 % ethanol extract of globe cucumber.

Keywords: globe cucumber, *Cucumis prophetarum*, cucumber, *Cucumis sativus*, cucurbitacins, *Aphis gossypii, Bemissia tabaci, Nezara viridula*

Introduction

Plant resistance to insects is used as integral part of insect pest management programs in many crops throughout the world. It may be used to enhance chemical control, resulting in reducing rates of insecticide application and ultimately less chemical placed in the environment (Smith, 1989). The cultivation of plants that are resistant to insects is a plant protection technique, has been used in several hundred years. Before the domestication of plants for agricultural purposes, those susceptible to insects died before they could produce seeds or before their damage seeds could germinate. Thus, resistant plants survived subject to the laws of adaptation and natural selection (Smith, 1989).

In addition, a large number of secondary metabolites in plants have a role in direct plant defence (Moraes *et al*., 2008). The variability in the amount of secondary metabolites in plants is clearly linked with genetic characteristics, although biotic and abiotic factors can provoke changes in this variability (Villagrasa *et al*., 2006; Oikawa *et al*., 2001, 2004).

On the other hand, plant resistance to insects is composed of the genetically inherited qualities that result in a plant of one cultivar or species being less damaged than is the susceptible one, which lacks these qualities. Plant resistance to insects is always relative, the degree of resistance is leased on comparison to susceptible plants that are more safely damaged under similar test conditions. There are different kinds of resistance: (i) false resistance, (ii) associational resistance (iii) plant morphology resistance, (iv) chemical defences (Smith, 1989).

In addition, allelochemicals may act as repellents during the olfactory orientation of an insect to a resistant plant or as feeding deterrents or feeding inhibitors when an insect tastes a resistant plant (Chapman, 1974; Macias *et al*., 2007).

The primary method used by plants to 'sense' the presence of insect herbivores has been shown to be insect oral secretions. Schittko *et al*. (2001) demonstrated that Manduca sexta regurgitant extensively modifies the tobacco wound response, eliciting jasmonic acid and ethylene bursts (Kessler and Baldwin, 2002), and confirmed the identity of the elicitors as fatty acid conjugates (Halitschke *et al*., 2001). Volicitin, a fatty acid conjugate present in lepidopteran oral secretions, also causes upregulation of expression of genes involved in the biosynthesis of both indole (Frey *et al*., 2000) and terpene volatiles in maize (Shen *et al*., 2000).

The cucurbitacins are most commonly associated with species in the *Cucurbitaceae*, and they have been detected in members of *Begoniaceae, Beassicaceae, Datiscacea, Desfontainaceae, Elaeocarpaceae, Rubiaceae, Scrophulariaceae* and *Sterculaceae* (Dinan *et al*., 2001).

On the other hand, cotton aphid (*Aphis gossypii*) attacks a wide variety of plants economically important like cucumber, avocado, banana, luffa, potato and tomato. Cotton aphid feed by sucking sap from their hosts and the heavy infestation cause a reduction in plant growth (Butani and Jotwani, 1983).

Cucumber and globe cucumber were the subject of this investigation, the plants of the family *Cucurbitaceae*, commonly known as gourd or melon. They are distributed throughout the world. Many of them are edible like cucumber while some are medicinal like globe cucumber or even ornamental plants. Plants of *Cucurbitaceae* family were found to contain a number of naturally related triterpenes, known collectively as cucurbitacins (Rizk, 1986).

Cucumber is trailing or climbing plant, rough-hairy and annual while globe cucumber is a wild medicinal plant (Baily, 1963) which is known as Shary. Hadaj, globe cucumber or cucumis. The fruits are medically used as emetic and purgative agents (Rizk, 1986).

In general, the present work was basically conducted to determine the relationship between the levels of both primary and secondary metabolites in the two plants and the degree of infestation of the prevailing insects. In addition, these relations could be used as chemical markers for plant resistance against insects. Also, it was checked the administration of some extracts as botanical insecticides to prevent the susceptible plants under study from insect infestation.

Materials and methods

Plant Materials

Seeds of globe cucumber (*Cucumis prophetarum* L.) were collected from Wadi Firran, Sinai, Egypt while the seeds of cucumber (*Cucumis sativus*) were obtained from the Ministry of Agriculture in Cairo. All the plants were identified by Prof. Dr. Loutfy Boulos, Department of Plant Taxonomy, National Research Centre, Dokki, Cairo, Egypt.

Susceptibility and resistance properties of two plants to insect infestation

The plants were cultivated at Kalubia governorate, Kafr El-Regalate village on the 5th of May in two successive seasons, 2005 and 2006. Total area (1/6 fedan) was divided into 12 plots for two plants. Each plot was 4x4 m and was surrounded by non-cultivated belt. Each plant was cultivated in six plots where the normal agriculture practices were done.

A sample of ten plants was examined randomly for counting the immature stages of cotton aphids (*Aphis gossypii* Glov.), white fly (*Bemissia tabaci* Gen.) and green stink bug (*Nezara viridula* L.). Samples were taken every two weeks during the growing season from the first of June to the first of August. Examination of insect infestation took place early in the morning to assure the stability of the pests and to avoid the adverse action of climatic conditions.

General phytochemical screening

Carbohydrates and/or glycosides content, flavonoids, sterols and/or triterpenes, coumarins, alkaloids, saponins, tannins, thio acids (glucosinolates) and cucurbitacins were detected in the two plant herbs according to the methods of Harborne (1998); Balbaa (1974); Attard and Scicluna-Spiteri (2001).

Determination of secondary plant metabolites

Total cucurbitacins were extracted and determined according the method of Attard and Scicluna-Spiteri (2001).

The colorimetric method of Folin-Denis as described by Swain and Hillis (1959) was employed for the determination of phenolic compounds in the leaves and stems of the two plants.

Total flavonoids was extracted from dried plant leaves (2 g) and determined according to Zhuang *et al*. (1992).

Determination of primary metabolites

Total soluble sugars (T.S.S.) were determined in the ethanolic extract of plant material, using the phenol-sulphuric acid method (Dubois *et al*., 1956). Total nitrogen (T.N.) was determined by micro Kjeldahl method (A.O.A.C., 1990). Acid hydrolysis was carried out according to the method of A.O.A.C. (1990) and both total and free amino acids (T.A.A. and T.F.A.A.) were determined according to the method of Rosein (1957). Conjugated amino acids (T.C.A.A.) were calculated by the difference between total amino acids and free amino acids according to A.O.A.C. (1990).

Determination of natural resistance using biochemical methods (Food Coefficients)

The results of biochemical analyses were used for the calculation of three food coefficients as described by Ciepiela *et al*. (1999) as follows: total soluble sugars over total nitrogen (T.S.S./T.N.), total soluble sugars over total free amino acids (T.S.S./T.F.A.A.) and total soluble sugars over total conjugated amino acids (T.S.S./T.C.A.A.).

Isolation and identification of cucurbitacins

Air dried powdered herbs (500 g) of two plants under investigation were separately extracted with 3 liters ethanol (95 %, v/v) at room temperature. The ethanolic extract was concentrated under reduced pressure at 40oC. The residue was suspended in water and extracted in succession with n-hexane, benzene, chloroform, ethyl acetate and water, respectively. The residual layers were evaporated under reduced pressure at 40oC. Every layer was checked for cucurbitacins, using phosphomolybdic acid reagent (2% of phosphomolybdic acid in absolute ethanol). The compounds expected to be cucurbitacins were streaked

on preparative thin-layer chromatography, scraped off and extracted with chloroform: methanol mixture (35 ml, 9:1.v/v). Then, they were re-purified again to insure purity. Each purified spot was re-crystallized from cold chloroform-methanol mixture (7:3 v/v) and re-chromatographed on preparative TLC plats and developed with one of the different following solvent systems, i.e., benzene: ethyl acetate (85:15 v/v), toluene: acetone (9:1 v/v) and chloroform: acetone (9:1 v/v). The RF values of the cucurbitacins in the samples were compared with RF values of the authentic samples. UV (240 1pc Shimatzu) and MS (EI+ Q1MS LMR UP LR, Masses: 50 >500) spectra were also performed to confirm the isolated cucurbitacins.

Bioassay Experiments

Preparation of extracts

Air dried powdered herbs (1 kg) of globe cucumber plant were extracted with 6 l of ethanol (95% v/v) at room temperature. The ethanolic extract was concentrated under reduced pressure at 40oC. The residue was suspended in water and extracted in succession with n-hexane, benzene, chloroform, ethyl acetate and water, respectively.

Ethanol, n-hexane, benzene, chloroform, ethyl acetate and water extracts of globe cucumber plant were diluted with distilled water using tween 80 (two drops served as an emulsifier). Three concentrations were prepared from each extract (1, 2 and 4 % w/v) and for each isolated cucurbitacins (0.05, 0.1 and 0.2% w/v) for bioassay experiments. Another treatment comprising distilled water, residue of the solvent and emulsifier served as control.

Insect culture of cotton aphid (Aphis gossypii Clov.)

A stock culture of cotton aphid was maintained on seedling cucumber under laboratory conditions of 20 ± 5oC, 65 ± 5% relative humidity (RH) and 12 hr photoperiod. The seedlings of cucumber used for the present experiments were grown to the 4-5 leaf stage in a mixture of clay (50%), sand (30%) and peat moss (20%), in 15 cm pots. Newly born apterous adults were transferred to cucumber seedlings previously planted in pots.

Insecticidal studies

The thin-film technique was used as follows. Each plastic Petri dish (5 cm in diameter) contained an upper circle hole (2 cm in diameter) and covered with muslin. The circle muslin was fixed on the hole using melted rubber to prevent the insect from escaping and for insect breathing. Apart of cucumber plant including one leaf and stem (1 month old) was placed into modified Petri dish and the stem was surrounded by a wetted piece of cotton to keep the leaves fresh and supplied daily with water during the period of experiments.

5 ml of each concentration were evenly applied on the modified Petri dish as well as the fresh leaves. The solvent was allowed to evaporate in a few min leaving a thin film of the plant extract or the isolated cucurbitacins.

Fifteen newly emerged adults were placed singly on the treated or control leaves in the modified Petri dishes as described previously. The mortality percentage was recorded after 24, 48, 72 and 96 hr and corrected using Abbot's formula (Abbot, 1925).

Biological studies

The effect of different extracts of globe cucumber plant on the various biological aspects of cotton aphid adults was studied. Each leaf was directly dipped for 5 seconds in each concentration and placed into modified Petri dish.

After 10 min, fifteen newly formed virgin apterous aphids were placed singly in each modified Petri dish on the previously treated leaves. The number of young off spring per each insect in the different concentrations as well as the control was counted daily.The whole longevity and the period of progeny production were recorded.

Control of cotton aphid using natural extracts under field conditions

The present field experiments were carried out at Bashtil village, Embaba, Giza governorate, during the year 2007.

Different extracts of globe cucumber plant which gave the highest mortality percentages as well as the highest effect on the biology of cotton aphids were applied again on cucumber plant to prevent it from insect infestation. Cucumber plants were cultivated in three replicates (3 x 3 m) and surrounded by non-cultivated belt, normal agriculture practices were done in all plants. All extracts were diluted with water using tween 80 as an emulsifier. Concentrations used were the lethal concentration (LC50) and its double.

A two-liter capacity hand sprayer (previously calibrated) was used to apply the different extracts on the aerial plant parts. Each extract was sprayed twice with an interval of 10 days. Before the first spray, the total numbers of cotton aphid were recorded in ten plants randomly. Infested samples were recorded from the different plots just before treatments and after 1, 3, 5, 7 and 9 days from each treatment.

The percentage infestation was estimated in the field based on the number of infested plants in relation to the total plants recorded. Also, the percentage reduction in infestation in each case was calculated using Hendreson and Tilton (1955) equation.

Statistical treatment of the data

Statistical analysis was carried out using CoStat version 3.03, a statistics computer program. The Duncan's test was used to determine significant differences between means of treatments (Duncan, 1956; Waller and Duncan, 1969 – commonly known method, not necessary to cite).

Results and discussion

Estimation of natural insect infestation

Tab. 1 shows the density of insects per plant. Cotton aphid population on cucumber was markedly increased after 6 weeks from sowing in the two seasons, reaching 10.4 and 13.3 insects per plant; the maximum number of cotton aphid was 44.2 insects / plant and 44.8 insects / plant in the 10th week during the first and the second seasons, respectively. In addition, the maximum number of white fly nymph per cucumber plant was 8.6 and 8.3 insects / plant after 10 weeks from sowing, during the first and the second seasons. The same trend was recorded in green stink bug where the maximum number reached 6.7 and 7.7 insects / cucumber plant after 14 weeks from sowing, during the first and the second season, respectively.

On the other hand, no cotton aphids and green stink bug were recorded on globe cucumber through the growth stages. In general, the distribution of insect populations on cucumber follows the sequence: cotton aphids > white fly nymph > adult green bug.

Distribution of secondary metabolites in plants

The present data in Tab. 2 showed the levels of cucurbitacins, flavonoids and phenolic compounds in the leaves and stems of two plants. The maximum level of both cucurbitacins and phenolic compounds were in globe cucumber and reached 4.23% in the 10th week during the first season and 1.43% in the 10th week during the second season, respectively. In general, globe cucumber contained greater levels of both cucurbitacins and phenolic compounds than cucumber while cucumber was contained greater levels of flavonoids. Leaves of all plants contained greater levels of secondary metabolites than stems. This phenomenon may be due to the accumulation of these compounds in the leaves after transportation through the stems.

It can be concluded that there was a relationship between insect infestation and cucurbitacins content in cucumber during the corresponding time. The population density of cotton aphid increased by increasing the concentration of cucurbitacins in leaves and stems of cucumber plants. Globe cucumber contained higher level of cucurbitacins and phenolic compounds in comparison with cucumber. It worth mentioning that, globe cucumber was not infested by cotton aphids. This phenomenon may be due to the high level of cucurbitacins and phenolic compounds accumulated during its growth stage. Hence, cucurbitacins content and phenolic compounds can be used as a chemical marker for insect resistance. In other words, these insects were repelled by globe cucumber due to the presence of high concentration of both cucurbitacins and phenolic compounds in comparison with cucumber plants.

On the other hand, the maximum flavonoids content occurred in cucumber at the same time where the population density of cotton aphids was high which means that these group of secondary plant metabolite had no biological activity either as anti-feedant or as repellant.

Many authors studied the relation between secondary metabolites and insect attack. Volatile hydrocarbons

Tab. 1. Natural insect infestation of cucumber and globe cucumber plants during two successive seasons

Globe cucumber						Cucumber						Sampling date after sowing (week)
2006			2005			2006			2005			
No. of green bug /plant	No. of white fly nymph /plant	No. of cotton aphid/ plant	No. of green stink bug / plant	No. of white fly nymph /plant	No. of cotton aphid/ plant	No. of green stink bug / plant	No. of white fly nymph /plant	No. of cotton aphid/ plant	No. of green stink bug / plant	No. of white fly nymph /plant	No. of cotton aphid/ plant	
0.0±0.0	0.0 ^{b}±0.0	0.0±0.0	0.0 ±0.0	0.0 ^{b}±0.0	0.0±0.0	0.0^{c}±0.0	0.0 ^{c}±0.0	0.0 ^{d}±0.0	0.0^{c}±0.0	0.0^{c}±0.0	0.0 ^{c}±0.0	2
0.0±0.0	0.0 ^{b}±0.0	0.0±0.0	0.0±0.0	0.0 ^{b}±0.0	0.0±0.0	0.0^{c}±0.0	7.7 ^{b}±0.4	13.3 ^{b}±0.6	0.0^{c}±0.0	8.3^{b}±0.5	10.4 ^{b}±0.3	6
0.0±0.0	1.3 ^{a}±0.0	0.0±0.0	0.0 ±0.0	0.0 ^{b}±0.0	0.0±0.0	5.2^{b}±0.5	8.3 ^{a}±0.6	44.8 ^{a}±2.8	5.4^{b}±0.3	8.6^{a}±1.0	44.2 ^{a}±0.6	10
0.0±0.0	0.0 ^{b}±0.3	0.0±0.0	0.0±0.0	2.1 ^{a}±0.3	0.0±0.0	7.7^{a}±0.8	0.0 ^{c}±0.0	8.7 ^{c}±0.5	6.7^{a}±0.3	8.6^{a}±0.5	10.0 ^{b}±0.5	14
0.0±0.0	0.0^{b}±0.0	0.0±0.0	0.0±0.0	0.0 ^{b}±0.0	0.0±0.0	0.0^{c}±0.0	0.0^{c}±0.0	0.0^{d}±0.0	0.0^{c}±0.0	0.0^{c}±0.0	0.0 ^{c}±0.0	18
--	0.081	--	---	0.073	--	0.207	0.141	0.591	0.152	0.276	1.322	LSD(5%)

Each value was following by standard error; the lower case letters indicate significant differences between insect infestation

148

Tab. 2. Total cucurbitacins, total flavonoids and total phenolic compounds in the leaves and stems of globe cucumber and cucumber based on dry weight during two successive seasons

Sampling date after sowing		Total cucurbitacins (%)				Total flavonoids (%)				Total phenolic compounds (%)			
		Globe cucumber		Cucumber		Globe cucumber		Cucumber		Globe cucumber		Cucumber	
Season	week	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
2005	2	1.88 c	0.91 ab	0.62 b	0.41 d	1.07 b	0.67 b	1.10 b	0.72 a	1.23 b	0.65 b	0.88 b	0.69 b
	6	3.17 b	1.43 ab	0.65 b	0.53 c	1.25 a	0.71 a	1.18 a	0.81 a	1.36 a	0.68 a	0.94 a	0.76 a
	10	4.23 a	2.14 a	1.84 a	0.92 a	1.08 b	0.67 b	1.20 a	0.85 a	1.20 c	0.63 c	0.95 a	0.79 a
	14	1.84 c	1.73 a	0.72 b	0.64 b	0.81 c	0.55 c	1.06 c	0.65 a	1.08 d	0.51 d	0.93 a	0.64 c
	18	1.81 c	1.52 ab	0.52 b	0.33 e	0.77 d	0.53 d	0.97 d	0.55 a	1.03 e	0.50 e	0.86 b	0.53 d
Mean		2.586	1.546	0.87	0.484	0.996	0.626	1.102	0.716	1.18	0.594	0.912	0.682
L.S.D. (1%)		0.478	0.658	0.12	0.04	0.011	0.007	0.03	0.39	0.005	0.006	0.04	0.04
2006	2	1.86 c	0.90 ab	0.54 d	0.38 d	1.18 b	0.66 b	1.18 b	0.69 a	1.33 c	0.60 c	0.93 b	0.61 c
	6	3.05 b	1.40 ab	0.67 c	0.54 b	1.28 a	0.73 a	1.26 a	0.74 a	1.39 b	0.65 a	1.00 a	0.68 b
	10	4.11 a	2.11 a	1.63 a	0.75 a	1.03 c	0.65 c	1.29 a	0.83 a	1.43 a	0.61 b	1.10 a	0.72 a
	14	1.80 c	1.70 a	0.86 b	0.53 b	0.68 d	0.52 d	1.25 a	0.63 a	1.13 d	0.48 d	0.98 a	0.54 d
	18	1.78 c	1.49 a	0.66 c	0.42 c	0.66 e	0.50 e	0.98 c	0.52 a	1.06 e	0.47 e	0.87 c	0.53 d
Mean		2.52	1.52	0.872	0.524	0.966	0.612	1.192	0.682	1.268	0.562	0.976	0.616
L.S.D. (1%)		0.495	0.704	0.11	0.05	0.013	0.008	0.041	0.40	0.006	0.007	0.05	0.04

emitted by the foliage of resistant plant may act to repel insects (Bordasch and Berryman, 1977). Similarly, volatiles from insect resistant rice cultivars repel feeding by the green rice leaf hopper (Mihm, 1985). The allelochemical compounds found most frequently to cause deterrence are alkaloids, terpene lactones and phenols. Green bug aphids are deterred for feeding by phenolics, procyanidin and p-hydroxyl benzaldehyde and dhurrin resistant sorghum cultivars (Dreyer *et al*., 1981).

Berenbaum (1990) outlined a coevolutionary scenario between coumarin containing Apiaceae and Caterpillars specializing on them. According to the investigations of Berenbaum (1992), the accumulation pattern of linear and angular furano coumarins explains to a large extend the distribution of *Depressaria pastinacella* larvae feeding on a population of attacked individuals of P. sativa displayed comparatively low concentration of furano coumarins in contact to the unattached ones. These reports unequivocally demonstrated the biological relevance of certain coumarin derivatives within the defense system of plants accumulating them.

Distribution of primary metabolites in two plants

Tab. 3 shows the concentrations of primary metabolites in two plants. The maximum level of total soluble sugars was in cucumber where reached 4.26% and 4.10% during the second week after sowing in the first and second season, respectively, and then gradually decreased in the first and second season, and again increased to reach 4.10% and 3.98% in the end of growth in the first and second season. The same trend was recorded for total nitrogen, total amino acids and conjugated amino acids, with differences in values.

On the other words, cucumber exhibited higher values of total soluble sugars, total nitrogen, and total free and conjugated amino acids than globe cucumber. The great fluctuation occurred with primary metabolites was correlated with the maximum peak of growth in which the plant uses the active soluble sugars, total nitrogen and total amino acids for its growth requirements. The levels of these primary substances were decreased at maturity. This is a natural phenomenon as the plants take its requirements and the high amounts were stored in the seeds or other plant parts to be used in other vital processes.

Total soluble sugar content was decreased with increasing cotton aphid's infestation in cucumber. globe cucumber contained the lowest level of total soluble sugars without inducing any infestation. This means that cotton aphids and green stink bugs didn't prefer soluble sugars in high concentration. With total nitrogen, cucumber contained the highest total nitrogen combined with the highest aphid infestation followed by globe cucumber. The same trend was observed with total and conjugated amino acids. That means aphid and green stink bugs prefer total nitrogen, total amino acids and total conjugated amino acids in high concentration for their growth. An opposite picture was observed with free amino acids.

Generally, globe cucumber had higher ratios of T.S.S over T.N, T.S.S over T.C.A.A. and T.S.S over T.F.A.A. than cucumber, in the two successive seasons.

The obtained data revealed that the high population density of the prevailing insect is correlated with the low ratio of T.S.S. / T.N. Similar results were observed in the ratio of T.S.S. / T.C.A.A. and could be added as indicator in the resistance of globe cucumber to insects.

The results of food coefficients were in parallel with the total cucurbitacins and total phenolic acids in globe cucumber in which their occurrence indicate the resistance to cotton aphids, white fly nymph and green stink bugs.

De-Ponti and Garretsen (1980) pointed out that the host resistance may result from the expression of genes conditioning the presence of chemical or physical at-

Tab. 3. Primary metabolites and food coefficient values of globe cucumber and cucumber leaves based on dry weight during two successive seasons

Sampling date after sowing		Total soluble sugars (%)		Total nitrogen (%)		Total amino acids (%)		Conjugated amino acids (%)		Total free amino acids (%)		T.S.S/T.N		T.S.S/T.F.A.A		T.S.S/T.C.A.A	
Season	week	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
2005	2	2.31 c	4.26 a	1.24 a	5.31 a	6.53 a	30.77 a	6.4 a	30.33 a	0.13 a	0.40 b	1.86 d	0.80 a	17.77 a	10.65 a	0.36 e	0.14 a
	6	2.34 b	3.85 b	1.20 b	5.05 b	4.31 b	26.50 b	4.17 b	26.11 b	0.14 a	0.53 b	1.95 c	0.76 a	16.71 b	7.26 b	0.56 c	0.15 a
	10	2.13 d	3.12 c	0.91 d	4.73 c	3.12 e	23.31 c	2.87 e	22.50 c	0.25 a	0.71 a	2.34 b	0.66 ab	8.52 c	4.39 d	0.74 b	0.14 a
	14	1.15 e	2.50 d	0.89 e	4.54 cd	3.24 d	21.79 d	2.88 d	21.05 d	0.36 a	0.73 a	1.29 e	0.55abc	3.19 d	3.42 e	0.40 d	0.12 b
	18	2.65 a	4.10 a	1.06 c	4.64 c	3.27 c	22.77 d	2.93 c	22.15 c	0.34 a	0.72 a	2.5 a	0.88 a	7.79 d	5.69 c	0.90 a	0.18 a
Mean		2.116	3.566	1.06	4.854	4.094	25.028	3.85	24.428	0.244	0.618	1.988	0.73	10.796	6.282	0.592	0.146
L.S.D. (1%)		0.0047	0.18	0.0069	0.13	0.0070	1.22	0.0069	0.38	0.48	0.14	0.005	0.13	0.06	0.08	0.0038	0.05
2006	2	2.41 b	4.10 a	1.19 a	5.25 a	5.89 a	30.12 a	5.71 a	29.67 a	0.18 a	0.47 b	2.02 c	0.78 a	13.39 a	8.72 a	0.42 e	0.14 a
	6	2.33 c	3.84 ab	1.15 c	5.01 b	5.55 b	24.66 b	5.32 b	24.13 b	0.23 a	0.57 b	2.03 b	0.77 a	10.13 b	6.73 b	0.44 c	0.16 a
	10	2.10 d	3.23 c	1.11 d	4.67 c	4.10 c	21.23 d	3.80 c	20.54 d	0.30 a	0.70 a	1.89 d	0.69 b	7.00 d	4.61 d	0.55 b	0.16 a
	14	1.20 e	2.43 d	0.93 e	4.42 d	3.16 e	20.30 d	2.77 e	19.53 e	0.39 a	0.75 a	1.29 e	0.55 c	3.08 e	3.24 e	0.43 d	0.12 a
	18	2.55 a	3.98 a	1.16 b	4.65c	3.39 d	23.14 c	3.10 d	22.47 c	0.29 a	0.70 a	2.20 a	0.86 a	8.79 c	5.69 c	0.82 a	0.18 a
Mean		2.118	3.516	1.108	4.8	4.418	23.89	4.14	23.268	0.278	0.638	1.886	0.73	8.478	5.798	0.532	0.152
L.S.D. (1%)		0.0049	0.17	0.0071	0.14	0.071	1.20	0.070	0.36	0.293	0.12	0.0071	0.11	0.70	0.06	0.0039	0.06

- I: Globe cucumber leaves; II: Cucumber leaves.
- The ratios T.S.S / T.N, T.S.S / T.F.A.A. and T.S.S. / T.C.A.A indicate the ratios between, total soluble sugars: total nitrogen, total soluble sugars: total free amino acids and total soluble sugars: conjugated amino acids, respectively.

Tab. 4. RF values, maximum UV absorption and the mass spectrum of different cucurbitacins isolated from globe cucumber and cucumber

Isolated cucurbitacins	Isolated from:	RF values			Maximum UV absorption (nm)	Mass spectrum (m/z)
		Benzene: ethyl acetate, (85:15)	Toluene: acetone, (9:1)	Chloroform: acetone, (9:1)		
Cuc. E	Globe cucumber (herbs) Cucumber (herbs)	0.312	0.116	0.712	248.6	539, 496, 479, 400, 383, 164, 113, 111, 96.
Cuc. B	Globe cucumber (herbs)	0.075	0.023	0.026	243	496, 190, 167, 149, 140, 127, 113, 95, 83, 60.
Cuc. D	Globe cucumber (herbs)	0.050	0.034	0.085	216	494,474,436,405, 190,167,149,140,127, 113,95,83, 60.
Cuc. I	Globe cucumber (herbs) Cucumber (herbs)	0.010	0.011	0.20	242.5	496, 479, 401, 314, 161, 113, 96.

tributes that interfere with the ability of an herbivore to utilize a plant compared to a plant not expressing those attributes. Resistance may also result from the absence of quantities essential for full utilization of a host plant by an herbivore. The relationships are illustrated by the effects of cucurbitacins on cucumber beetles (*Diabrotica spp*.) and two–spotted spider mites (*Tetranychus urticae* Koch). Cucurbitacins are tetracyclic triterpenoid compounds that act as powerful feeding stimulants for cucumber beetles. Also, cucurbitacins confer antibiosis resistance to spider mites. The absence of cucurbitacins in cucumber is controlled by the recessive bi-genes. Cucumber plant, lacking cucurbitacins, are highly susceptible to mites and resistant to beetles. Whereas, those containing cucurbitacins (Bibi or BiBi) are resistant to mites and susceptible to beetles.

In addition, Kamel and El-Gengahi (2008) found that bitter candytuft was more resistant to insect attack than broccoli, due to the higher level of glucosinolates and the presence of cucurbitacins. Also, the higher ratio of T.S.S. over T.N. and T.S.S. over T.C.A.A. in bitter candytuft than in broccoli could be added as indicator in the resistance of bitter candytuft to insect attack.

Identification of each cucurbitacin from two plants

Cucurbitacin E

This compound was isolated from the two plants, the RF values of this compound using the three solvent systems as mentioned before were 0.312, 0.116 and 0.712. The maximum absorption of compound 1 was $\lambda^{Max}_{Chloroform}$ 248.6. Mass spectrum of this compound shows a molecular ion at m/z = 539 corresponding to a molecular formula (C 32 H 43 O 7). The peaks at m/z = 496 (9.56 %) and 479 (2.5%) were due to the loss of CO-CH3 and OH group, respectively. The peaks at m/z = 400 and 383 may be due to loss of side chain by fission of the double bond between the carbon atoms 23 and 24. A very intense and characteristic peak, often the base peak was found at m/z = 96 (C6 H8 O).It is worth noting that the loss of either an OH or acetate group and leaving isopropenyl group was also occurred in compound I (Curtis and Meade, 1971). The mass spectrum indicates the presence of peaks at m/z = 164 and 113, characteristic for C6H14O2 , the peak at m/z = 111 might be corresponding to the formula C6H9O2. The strong peak at m/z = 96 (100%) suggests the occurrence of C25 acetyl derivative of this compound.

The aforementioned spectral data and RF values indicate that the chemical structure of this compound is cucurbitacin E.

Cucurbitacin B

This compound was isolated from globe cucumber only, the RF values of this compound were 0.075, 0.023 and 0.026 in using the three solvent systems mentioned before. The maximum absorption of this compound was $\lambda^{Max}_{Chloroform}$ 243.0. Mass spectrum of this compound shows a molecular ion at m/z = 496 corresponding to molecular formula (C30 H42O7). The peaks at m//z = 190,167,149,140,127,113,95,83, and 60 are characteristic of the saturated ring A and unsaturated ring.

From the aforementioned spectral data and RF values of this compound suggested that the chemical structure is cucurbitacin B.

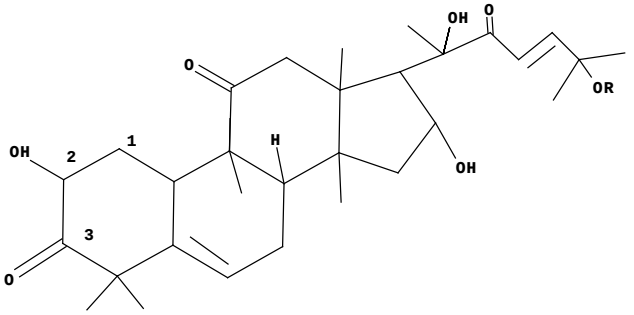
Cucurbitacin D

This compound was isolated from globe cucumber only. The RF values of this compound were 0.050, 0.034 and 0.085 in using the three solvent systems mentioned before. The maximum absorption of cucurbitacin D was MaxChloroform 216 nm. The mass spectrum of cucurbitacin D shows a molecular ion at m/z = 494 corresponding to molecular formula (C30 H44O7). The peak at 474 is appeared due to loss of OH group. The peak at m/z = 436 and 405 may be due to loss of side chain (C-C-C and C-C-C). The peaks at m//z = 190, 167, 149, 140, 127, 113, 95, 83, and 60 are characteristic of the saturated ring A and unsaturated ring.

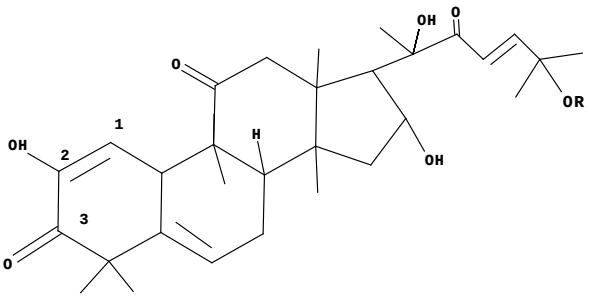
In general, the mass fragments of this compound gave the same MS fragment pattern of cucurbitacin D authentic sample and with those reported in literature (Stuppner *et al*., 1990; Hatam *et al*., 1989; Schenkel *et al*., 1992).

Cucurbitacin I

This compound was isolated from two plants under study, the RF values were 0.010, 0.011 and 0.20, in using the three solvent systems mentioned before. The



Cucurbitacin B, R=COCH3; Cucurbitacin D, R= H



Cucurbitacin E, R= $COCH_3$; Cucurbitacin I, R=H

Scheme (1): Chemical structure of cucurbitacins isolated from globe cucumber and cucumber plants.

Tab. 5 Corrected mortality percentages of cotton aphid adults treated with different concentration of globe cucumber extracts and isolated cucurbitacins

Plant extracts and isolated cucurbitacins	Concentration (% W/V)	Corrected mortality after the indicated period (%)				Corrected cumulative mortality (%)
		24 h	48 h	72 h	96 h	
Ethanol	1	13.3	6.7	6.7	6.7	33.3
	2	13.3	26.7	13.3	26.7	80.0
	4	26.7	26.7	26.7	13.3	93.4
n-Hexane	1	6.7	6.7	6.7	0.0	20.1
	2	6.7	6.7	6.7	0.0	20.1
	4	6.7	6.7	6.7	6.7	26.8
Benzene	1	13.3	13.3	0.0	0.0	26.6
	2	13.3	6.7	6.7	13.3	40.0
	4	13.3	13.3	13.3	13.3	53.2
Chloroform	1	13.3	6.7	6.7	0.0	26.7
	2	13.3	6.7	6.7	6.7	33.4
	4	13.3	13.3	13.3	13.3	53.2
Ethyl acetate	1	6.7	6.7	6.7	0.0	20.1
	2	6.7	6.7	6.7	6.7	26.7
	4	6.7	6.7	6.7	6.7	26.7
Water	1	6.7	6.7	0.0	0.0	13.4
	2	6.7	6.7	0.0	0.0	13.4
	4	6.7	6.7	6.7	0.0	20.1
Cucurbitacin E	0.05	0.0	0.0	0.0	6.7	6.7
	0.1	0.0	0.0	6.7	6.7	13.4
	0.2	6.6	20.0	13.3	20.0	60.0
Cucurbitacin B	0.05	0.0	0.0	0.0	6.7	6.7
	0.1	0.0	0.0	6.7	6.7	13.4
	0.2	6.7	6.7	13.3	13.3	40.0
Cucurbitacin I	0.05	0.0	0.0	0.0	6.7	6.7
	0.1	0.0	0.0	0.0	6.7	6.7
	0.2	0.0	0.0	0.0	13.3	13.3
Cucurbitacin D	0.05	0.0	0.0	0.0	0.0	0.0
	0.1	0.0	0.0	0.0	6.7	6.7
	0.2	0.0	0.0	0.0	6.7	6.7
L.S.D. (5%)						0.0395

Tab. 6. Effect of globe cucumber extracts on the development of cotton aphid

Globe cucumber extracts	Concentration (% W/V)	Adult instars		
		days± S.E.		Fecundity/insect ±S.E.
		Period of progeny production	Longevity	
Ethanol	1	6.7±0.7	7.4±0.6	18.4±1.8
	2	5.3±0.6	7.2±0.7	17.2±1.7
	4	4.6±0.2	6.0±0.8	9.4±1.6
n-Hexane	1	8.3±0.4	9.4±0.7	27.4±1.6
	2	8.1±0.5	9.2±0.6	26.5±1.4
	4	8.0±0.4	9.1±0.4	23.4±1.4
Benzene	1	8.6±0.4	9.4±0.7	22.4±1.4
	2	8.1±0.7	9.2±0.8	21.7±1.5
	4	7.4±0.1	9.1±0.5	19.3±1.6
Chloroform	1	8.1±0.3	7.4±0.6	19.4±1.8
	2	7.2±0.4	7.2±0.7	18.2±1.7
	4	6.1±0.4	7.0±0.8	12.4±1.6
Ethyl acetate	1	8.6±0.7	9.3±0.7	28.4±1.4
	2	8.2±0.6	8.7±0.4	27.4±1.3
	4	8.1±0.5	7.8±0.4	25.9±1.8
Water	1	9.7±0.4	10.2±0.9	29.0±1.4
	2	9.3±0.3	9.8±0.8	28.7±0.4
	4	9.1±0.1	9.7±0.7	28.4±0.2
Control		10.3±0.8	11.9±1.2	29.3±2.7
L.S.D. (5%)		0.117	4.579	1.855

maximum absorption of compound III was $\lambda_{Max}^{Chloroform}$ 242.5. Mass spectrum of this compound shows molecular ion at m/z = 496 corresponding to molecular formula ($C_{30}H_{40}O_7$). The peak at 479 appeared due to loss of OH group. The peak at m/z = 401 may be due to loss of side chain. In this case, the very intense and characteristic peak, often the base peak was found at m/z = 96 (C_6H_8O). The peaks at m/z = 161and 113 were related to (C9H14O2) and (C6H14O2), respectively. The strong peak at m/z = 96 (100%) suggesting that it was the C25 derivative of cucurbitacin I. The peak at m/z = 314 is due to the cleavage between C5 and C6 after rearrangement of the 11 keto.

The aforementioned spectral data and RF values indicate that the chemical structure of this compound is cucurbitacin I.

CucurbitacinB,R=COCH3 CucurbitacinE,R= COCH3

Cucurbitacin D, R= H Cucurbitacin I, R=H

Insecticidal activity

Tab. 5 presents the insecticidal activity of various extracts of globe cucumber and different isolated cucurbitacins. Ethanol extract induced the most toxic effect followed by chloroform extract when compared with all plant extracts. This can be described on the basis that ethanol extract contains cucurbitacins, flavonoids and phenolic compounds which is more toxic than other secondary metabolites on the cotton aphid.

Major cucurbitacins were isolated from the two plants to study their insecticidal activity. The data revealed that

Tab. 7. Efficiency of spraying cucumber with different concentrations of globe cucumber extracts on the cotton aphid

	Conc. (%)	No. of aphids	Number of living aphids/10 plants at indicated days after spraying								Mean	Total reduction of infestation (%)
			1st spray				2nd spray					
Ethanol extract			1	3	5	7	11	13	15	17		
	2	96.4	92.1	82.4	60.3	53.3	22.1	0.0	0.0	0.0	38.77	74.0
	4	88.4	71.0	61.3	28.1	22.2	0.0	0.0	0.0	0.0	22.82	82.0
Control		130.6	135.1	143.2	166.2	176.2	184.3	190.2	185.7	160.4	167.66	---

the corrected mortality percentage increased by increasing the concentration, where cucurbitacin E at 0.2 % level induced 60.0 % mortality. Cucurbitacin B at 0.2% concentration induced 40.0% mortality. Lower insecticidal activity was revealed when cucurbitacin I and cucurbitacin D were used (13.3% and 6.7%, respectively).

In addition, the relationship between chemical structure of cucurbitacins and its insecticidal activity was studied. It could be seen that cucurbitacin E was more toxic than B, D and I. Four cucurbitacins contain the same chemical structure except for R group and double bond between the positions 1 and 2. Cucurbitacin E and B contain the acetyl group while cucurbitacin E contains also a double bond between the carbon atoms in the positions 1 and 2. The presence of the double bond possessed an increase in its biological activity. Lower insecticidal activity was recorded with cucurbitacin D due to the absence of both double bond in the positions 1 and 2 besides the absence of acetyl group.

It could be concluded that cucurbitacin E was the most toxic one followed by cucurbitacin B, cucurbitacin I and finally cucurbitacin D. These finding suggest that the acetyl group is very important for the toxicity and the toxicity of the compound which contains acetyl group is increased with the presence of a double bond between the carbon atoms in the positions 1 and 2.

Dinan *et al*. (2001) found that many insect species are deterred or killed by the presence of cucurbitacins in the diet, although diabroticite beetles (which specialize on cucurbits) are attracted by low to moderate concentrations.

In addition, Gillespie *et al*. (2003) assumed that cucurbitacin feeding and sequestration in root worm leaf beetles is remnant of an ancient association between the Luperini (*Coleoptera*) and *Cucurbitaceae*. Under this premise, root worms that didn't develop on cucurbits but undergo pharmacophagous forays for cucurbitacins were thought to do supplement novel host diets that lake these bitter compounds.

Effect of the tested extracts on the adult instars of cotton aphid

The results of these experiments are shown in Tab. 6. It is obvious that the period of progeny production of cotton aphid was highly affected by ethanol extract of globe cucumber at 4% where the period of progeny production reached 4.6 days per insect. Water extract of globe cucumber had a lower biological activity where the period of progeny production was nearly similar to the control experiment (9.1 days in comparison with 10.3 days in control). The effect of different extracts on the longevity of the adult aphid was studied. The data revealed that the longevity decreased with ethanol extract at 4% till reached 6.0 days. In all different extracts, the longevity was in parallel with the concentration. Ethanol extract of globe cucumber at 4% was unsuitable for progeny as the majority of nymph were laid on the untreated leaves while few number of nymphs were laid on treated ones. An opposite picture was observed with water extract where the number of deposited nymphs was nearly similar to the control experiment.

Here again, the ethanol at various concentrations of globe cucumber induced the highest mortality percentages as well as affected on cotton aphid's biology. It seems that ethanol as a solvent extracted all cucurbitacins, phenolic compounds and flavonoids from globe cucumber plants and induced the highest cotton aphid mortality. In the meantime, the chloroformic extract contains the cucurbitacin compounds and induced also the highest cotton aphid mortality and affected on aphid's biology.

Several authors studied the effect and the role of different plant extracts on insects. For instance, Dimetry and El-Hawary (1995) studied the activity of different alcohol extracts of citrullus, curcuma, nicandra herb against cowpea aphid (*Aphis craccivora* Koch). They found that curcuma ethanol extract was the most toxic against cowpea aphid followed by citrullus seed and nicandra. Also, nymphal treated with curcuma prolonged the duration of the nymphs.

On the other hand, Ferry *et al*. (2004) reported that many insects are able to detoxify potentially secondary metabolites, using cytochrome P540 monooxygenases and glutathione S-transferases. These enzymes are induced by exposure to toxic plant secondary compounds; for instance, xanthotoxin, as an example for furanocoumarin compounds induced P450 expression in corn earworm.

Control of cotton aphid and cabbage aphid using natural extracts under field conditions

From Tab. 7, it was noticed that ethanol extract of globe cucumber at 4% concentration kept cucumber plants healthy with 82.0 % reduction of cotton aphid.

Schulz *et al*. (1997) mentioned that different Neem Azal formulations (1% Azadirachtin- A, 51% plant oils) were effective on aphids in laboratory and field trials. Neem Azal sprays to broad bean and apple trees resulted in significant reduction in number of bean aphid (*Aphis fabae Scop*) and rosy apple aphid (*Dysaphis plantaginea*).

Finally, Kamel and El-Gengaihi (2008) found that water extract after autolysis of bitter candytuft was the most effective extracts on the cabbage aphid's biology and could bw used as botanical insecticide to prevent broccoli from insect attack.

Conclusions

It can be concluded from all the obtained data that there was a relation between insect infestation and secondary and primary metabolites as well as food coefficients parameters.

No cotton aphids and green stink bug were recorded on globe cucumber through the growth stages. This phenomenon due to the highest level of cucurbitacins, phe-

nolic compounds and the highest ratios of T.S.S over T.N, T.S.S over T.C.A.A. and T.S.S over T.F.A.A. than cucumber, in the two successive seasons. In addition, the maximum flavonoids content occurred at the same time where the population density of cotton aphids was high on cucumber which means that this group of secondary plant metabolite had no biological activity on insects.

Ethanol extract of globe cucumber induced the most toxic effect on cotton aphids followed by chloroform extract, when compared with all plant extracts.

Major cucurbitacins were isolated from the two plants to study their insecticidal activity (%). Cucurbitacin E (0.2%) induced 60.0 % mortality followed by cucurbitacin B (40.0%). Lower insecticidal activity was revealed when cucurbitacin I and cucurbitacin D were used. There are a relation between the chemical structure of cucurbitacins and their biological activity.

Finally, ethanol extract of globe cucumber at 4% kept cucumber plants healthy, with 82.0% reduction of cotton aphid, and this extract could be used as botanical insecticide to prevent cucumber from insect attack.

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