



Effect of treated cucumber by cytokinin hormone on the infestation of *Aphis gossypii* (Hemiptera: Aphididae) and *Tetranychus urticae* (Acari: Tetranychidae) under glasshouse

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Abstract:

Cucumber (*Cucumis sativus* L.) is considered as one of the most important fresh consumed vegetables in Egypt and it is used to cultivate under open field conditions and greenhouse. The aim of this research work is to study effect of treated cucumber plants *C. sativus* by different concentrations of cytokinin hormone CKs (25 ppm, 45ppm and 65 ppm) on the infestation by *Aphis gossypii* Glover (Hemiptera: Aphididae) and *Tetranychus urticae* Koch. (Acari: Tetranychidae) at two locations in Giza and Qalubiya Governorates during 2018 season under glasshouse conditions. Also, this research work was carried out to study effect of treated cucumber plants by the same concentrations of the same hormone on the morphological characteristics and internal components of treated cucumber plants. Results obtained showed that cucumber plants treated with small concentration of CKs (25 ppm) were lower infestation by both *A. gossypii* and *T. urticae* compared to control. While cucumber plants treated with medium concentration of CKs (45ppm) were had no significant differences in the infestation by both *A. gossypii* and *T. urticae* compared to control. On the other hand cucumber plants treated with high concentration of CKs (65ppm) were higher infestation by both two pests compared to control. Also, results obtained showed that treated cucumber plants with small concentration of CKs improved morphological characteristics and internal components of these plants compared to control, while treated cucumber plants with medium concentration of the hormone had no significant differences on the morphological characteristics and internal components of these plants compared to control. Treated cucumber plants with high concentration of the same hormone had badly effect on the morphological characteristics and internal components of these plants compared to control.

Introduction

Cucumber (*Cucumis sativus* L.) considers one of the most important vegetables crops in Egypt and all over the

world which cultivated in the open field and under glasshouse conditions. Also, its cultivated area increased gradually during the last years, especially in the new reclaimed

areas for purposes local consumption and exportation to the foreign markets (Hanafy, 2004). Cucumber crop infested with large scale of different insects such as *Aphis gossypii* Glover (Hemiptera: Aphididae). It is considered one of the most damaging insects infesting vegetables crops either in the open field or under greenhouse conditions (Adriaan *et al.*, 2013). Also, *A. gossypii* beside its effects on leaves and fruits, transmit cucumber mosaic virus (CMV). It causes a serious disease of narrow-leafed lupin (Deborah *et al.*, 2012). Marabi *et al.* (2017) stated that aphids cause sporadic yield losses due to direct feeding damage. They reported that the aphid *A. gossypii* a harmful pest on most vegetables crops and causes direct damage by reducing plant vigor and indirect damage by honeydew secretion and transmission of several viruses. Red spider mite, *Tetranychus urticae* Koch. (Acari: Tetranychidae) also is considered as one of the most important pests infesting cucumber plants both in the open field and under glasshouse conditions. *T. urticae* is a species of plant-feeding mite generally considered to be a serious pest on cucumber plants and other vegetables crops. It is the most widely known member of the family Tetranychidae, it is a serious pest on cucumber plants under glasshouse conditions (Derek, 2013).

Cytokinin hormone (CKs) is considered one of the most famous and important phytohormone. There are more studies showed the important role of this hormone for growth regulators plants and its role for change morphological and physiological plant adjectives when used by different concentrations. Marcel (2015) reported that cytokinins are plant hormones that have among many other functions on the morphological and physiological characteristics on different plants. Also, Giron *et al.* (2013) stated that phytohormone cytokinins play important roles in regulating plant growth and defense against many dangerous pests. In India, Srivastava and

Srikant (2015) studied effect of cytokinin hormone on photosynthesis, alkaloid and other parameters in *Papaver somniferum* L. and studied the influence of different foliar application of cytokinin hormone on growth, CO₂, exchange rate, total chlorophyll, plant height and weight and fresh and dry weight of the leaves and shoots.

The aim of this research work is to study effect of treated cucumber plants var. *Cucumis sativus* L. by different concentrations of cytokinin hormone CKs (25 ppm, 45 ppm and 65 ppm) on the infestation by *A. gossypii* and *T. urticae* at two locations, Giza Governorate and Qalubia Governorate during 2018 season under glasshouse conditions. As well as, this research work was carried out to study effect of treated cucumber plants by the same concentrations of the same hormone on the morphological characteristics and internal components of treated cucumber plants.

Materials and methods

1. Experimental design:

This study was conducted on cucumber plants (*C. sativus*) at two locations, Giza and Qalubia Governorates during 2018 season. Plants were cultivated at both locations at the same time in a timely manner for the cultivation of cucumber (early summer planting) during period (February – April). At both locations we used two glasshouses, each glasshouse divided into four parts, three parts for the three treatments (Three concentrations of CKs) and the fourth part left as control. In the first treatment we immersion cucumber seedlings in low concentration of CKs (25ppm) for period 24 hour before cultivated. In the second treatment we immersion seedlings in medium concentration of CKs (45ppm) for period 24 hour before cultivated. In the third treatment we immersion seedlings in high concentration of CKs (65ppm) for period 24 hour before cultivated. Lastly, in the fourth treatment we did not immersion cucumber seedlings in any hormone before cultivated, this treatment used as control. These

seedlings cultivated under glasshouse conditions at both two locations at the same time. Then it was conducted all agricultural operations in a manner quite similar at two locations. The normal and recommended agricultural practices were applied, also no chemical control against insects were used during the whole experimental period. An artificial infestation with *A. gossypii* and *T. urticae* were done at the first and second glasshouses, respectively at the same time in the two locations. It is proven accurate observations of the infestation by the two pests number in all plants biweekly. Directly counting was done biweekly during the seasons at both locations allover plants.

2.Laboratory design:

Laboratory studies were carried out to study effect of treated cucumber plants by different concentrations of cytokinin hormone (CKs) on the morphological characteristics of treated cucumber plants such as root length (cm), shoot length (cm) and plant height (cm) and comparing these characteristics with control plants , did not treat with any hormone. Also, these laboratory experiments were carried out to study effect of treated cucumber plants by the same concentrations of cytokinin hormone (CKs) on the internal components of treated cucumber plants such as total protein (mg/g), total sugar (mg/g), starch (mg/g), amino acids (mg/g) and total phenols (mg/g), and comparing these concentrations with control plants did not treat with any hormone.

3. Determination of protein banding pattern:

3.1.Total protein extraction:

Total proteins were extracted from 0.5 kg fresh tissue of cucumber leaves. The tissues were ground in liquid nitrogen with a mortar and pestle. Then few mls of tris buffer extraction were added (1:2, tissue: buffer). The medium of extraction contained tris-HCL buffer (0.1mM tris, pH 7.5, 4m M B-mercapto ethanol, 0.1m M EDTA-Na₂, 10m M KCl and 10m M MgCl₂). The crude homogenate was centrifuged at 10.000xg for

20min. The supernatant was used for gel analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970).

3.2. Loading on a gel:

3.2.1. Gel preparation:

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 12.5% acrylamide and 0.8% bis-acrylamide running gel consisting of 0.375 M Tris-HCl (pH 8.8) and 0.1% SDS. Stacking gel (10 mm) was made using 4.5% acrylamide containing 0.8% bis-acrylamide in 0.125 M Tris-HCl (pH6.8) and 0.1% SDS. The electrophoresis buffer contained 0.025 M Tris-HCl, 0.19 glycine and 0.1% SDS. The samples were homogenized in 0.12M Tris-HCl (pH 6.8), 0.4 SDS, 10 B-mercaptoethanol, 0.02% bromophenol blue and 20% glycerol. The samples were then heated for 3min. in a boiling water bath before centrifugation. The gel was run under cooling at 90v for the first 15min., then 120v the next 0.5 hour and finally 150v for the remaining 1.5 hour (Sheri *et al.*, 2000).

3.2.2. Sample loading:

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

3.2.3. Electrophoresis conditions:

The running buffer was poured into pre-cooled (4°C) running tank. The running buffer was added in the upper tank just before running, so that the gel was completely covered. The electrodes were connected to power supply adjusted at 100 v until the bromophenol blue dye entered the resolving gel, and then increased to 250v until the bromophenol blue dye reaches the bottom of the resolving gel.

3.2.4.Gel analysis:

Gels were photographed using a Bio-Rad gel documentation system. Data analysis was obtained by Bio-Rad Quantity one Software version 4.0.3.

4. Statistical analysis:

In these experiments, effect of treated cucumber plants by different concentrations of (CKs) on the infestation by *A. gossypii* and *T. urticae* and effect of treated cucumber plants by different concentrations of (CKs) on the morphological characteristics and the internal components of cucumber plants were subjected to analysis of variance (ANOVA) and the means were compared by L.S.D. test at 0.05 level, using SAS program (SAS Institute, 1988).

Results and discussion

1. Effect of treated cucumber plants by different concentrations of (CKs) on the infestation by *Aphis gossypii* and *Tetranychus urticae*.

2. In Giza Governorate :

Data tabulated in Table (1) show the population fluctuation of *A.gossypii* and *T. urticae* on cucumber plants which treated by different concentrations of cytokinin hormone (CKs) in Giza Governorate during 2018 season. Data showed that cucumber plants which treated by low concentration of CKs

(25ppm) were lower infestation by both *A. gossypii* and *T. urticae* compared to control, cucumber plants which treated by medium concentration of CKs (45ppm) were had no significant differences in the infestation by the two pests compared to control and cucumber plants which treated by high concentration of CKs were higher infestation by both the two pests compared to control. Whereas mean number of aphid in control was (37.9 aphid/leaf), mean number in the first treatment (low concentration of CKs) was (18.4 aphid/leaf), mean number in the second treatment (medium concentration of CKs) was (35.6 aphid/leaf) and mean number of aphid in the third treatment (high concentration of CKs) was (40.6 aphid/leaf). Also, as the same trend, the mean number of *T. urticae* in control was (24.5 pest/leaf), mean number in the first treatment was (13.2 pest/leaf), the mean number in the second treatment was (22.6 pest/leaf) and the mean number in the third treatment was (28.9 pest/leaf).

Table (1): Population fluctuation of *Aphis gossypii* and *Tetranychus urticae* on cucumber plants which treated by different concentrations of cytokinin hormone (CKs) in Giza Governorate during 2018 season.

Date	<i>Aphis gossypii</i>				<i>Tetranychus urticae</i>			
	25 ppm	45 ppm	65 ppm	Control	25 ppm	45 ppm	65 ppm	Control
1/2/2018	10.7 ^c	26.5 ^a	29.8 ^b	28.3 ^a	6.3 ^b	15.7 ^a	19.3 ^b	17.2 ^a
8/2/2018	12.6 ^c	28.2 ^a	33.4 ^c	31.2 ^a	8.4 ^c	18.9 ^a	21.7 ^b	19.4 ^a
15/2/2018	14.2 ^b	31.7 ^b	35.1 ^c	33.7 ^a	10.8 ^c	19.2 ^a	24.3 ^b	21.5 ^a
22/2/2018	17.4 ^c	33.1 ^a	37.7 ^b	35.6 ^a	12.3 ^b	21.6 ^a	28.5 ^c	22.3 ^a
1/3/2018	19.7 ^c	35.9 ^b	39.8 ^b	38.8 ^a	13.2 ^c	23.8 ^a	31.1 ^c	24.5 ^a
8/3/2018	21.4 ^b	38.2 ^b	41.7 ^c	40.2 ^a	15.8 ^b	24.7 ^a	33.8 ^b	25.9 ^a
15/3/2018	23.1 ^c	40.8 ^a	43.8 ^b	42.4 ^a	17.2 ^c	25.5 ^b	35.2 ^c	27.7 ^a
22/3/2018	24.7 ^c	41.5 ^a	45.9 ^b	43.4 ^a	18.3 ^c	27.9 ^b	36.4 ^c	29.5 ^a
29/3/2018	25.3 ^c	43.2 ^a	48.7 ^c	45.5 ^a	20.5 ^c	29.1 ^a	37.5 ^b	31.3 ^a
5/4/2018	22.6 ^c	40.3 ^b	45.4 ^b	41.8 ^a	17.7 ^b	25.9 ^a	30.1 ^b	28.7 ^a
12/4/2018	18.2 ^c	37.2 ^a	43.5 ^b	39.2 ^a	13.8 ^c	22.3 ^b	28.4 ^b	25.5 ^a
19/4/2018	15.8 ^b	34.2 ^b	42.6 ^b	37.9 ^a	10.1 ^c	20.5 ^b	26.3 ^b	23.4 ^a
26/4/2018	13.2 ^c	31.5 ^a	39.8 ^c	34.3 ^a	7.3 ^c	19.1 ^a	23.8 ^c	21.3 ^a
Total	238.9	462.3	527.2	492.3	171.7	294.2	376.4	318.2
Mean	18.4	35.6	40.6	37.9	13.2	22.6	28.9	24.5
F(0.05)	732.25				845.63			
LSD	1.025				1.032			

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

1.2. In Qalubiya Governorate:

Data tabulated in Table (2) showed the population fluctuation of *A.gossypii* and *T. urticae* on cucumber plants which treated by

different concentrations of cytokinin hormone (CKs) in Qalubiya Governorate during 2018 season.

Table (2): Population fluctuation of *Aphis gossypii* and *Tetranychus urticae* on cucumber plants which treated by different concentrations of cytokinin hormone (CKs) in Qalubiya Governorate during 2018 season

Date	<i>Aphis gossypii</i>				<i>Tetranychus urticae</i>			
	25 ppm	45 ppm	65 ppm	Control	25 ppm	45 ppm	65 ppm	Control
1/2/2018	9.7 ^c	24.7 ^a	29.6 ^c	26.3 ^a	5.3 ^c	13.5 ^a	19.5 ^b	15.7 ^a
8/2/2018	10.6 ^c	27.5 ^b	31.5 ^b	29.2 ^a	7.4 ^c	15.7 ^b	20.3 ^c	17.3 ^a
15/2/2018	11.2 ^b	29.3 ^a	33.7 ^c	30.7 ^a	8.8 ^c	17.5 ^b	21.3 ^b	19.5 ^a
22/2/2018	13.4 ^c	31.2 ^a	35.9 ^b	33.6 ^a	10.2 ^b	20.6 ^a	23.5 ^c	21.3 ^a
1/3/2018	15.2 ^c	33.7 ^b	37.5 ^b	36.8 ^a	11.5 ^c	21.8 ^a	26.6 ^b	23.5 ^a
8/3/2018	17.5 ^b	35.9 ^a	39.8 ^c	38.2 ^a	13.7 ^b	23.1 ^b	28.8 ^b	25.4 ^a
15/3/2018	18.4 ^c	37.3 ^a	41.5 ^c	39.4 ^a	15.2 ^c	25.7 ^a	30.2 ^c	27.2 ^a
22/3/2018	20.3 ^c	38.7 ^b	43.8 ^c	41.4 ^a	17.3 ^c	27.5 ^a	33.4 ^c	28.5 ^a
29/3/2018	22.5 ^b	39.5 ^b	45.7 ^c	43.5 ^a	19.5 ^c	28.3 ^a	35.5 ^b	29.3 ^a
5/4/2018	18.6 ^c	37.4 ^a	43.1 ^b	39.8 ^a	16.7 ^b	24.9 ^a	30.2 ^c	26.7 ^a
12/4/2018	14.2 ^c	35.8 ^b	41.5 ^b	36.2 ^a	12.8 ^c	21.3 ^b	28.1 ^c	23.5 ^a
19/4/2018	12.8 ^b	33.2 ^a	38.6 ^c	34.9 ^a	8.1 ^c	19.5 ^a	26.3 ^b	20.4 ^a
26/4/2018	10.2 ^c	30.2 ^a	35.8 ^b	32.3 ^a	5.3 ^b	15.1 ^a	23.8 ^c	17.3 ^a
Total	194.6	434.4	498.0	462.3	151.8	274.5	347.5	295.6
Mean	15.0	33.4	38.3	35.5	11.7	21.1	26.7	22.7
F(0.05)	645.32				765.21			
LSD	1.043				1.035			

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

Data showed that cucumber plants treated by low concentration of CKs (25ppm) were lower infestation by both *A. gossypii* and *T. urticae* compared to control, cucumber plants treated by medium concentration of CKs (45ppm) were had no significant differences in the infestation by both the two pests compared to control and cucumber plants treated with high concentration of CKs were higher infestation by both two pests compared to control. Whereas mean number of aphid in control was (35.5 aphid/leaf), the mean number in the first treatment (low concentration of CKs) was (15.0 aphid/leaf), the mean number in the second treatment (medium concentration of CKs) was (33.4 aphid/leaf) and the mean number of aphid in the third treatment (high concentration of CKs) was (38.3 aphid/leaf). Also, as the same trend, the mean number of *T. urticae* in the control was (22.7 pest/leaf), the mean number in the first treatment was (11.7 pest/leaf), the

mean number in the second treatment was (21.1 pest/leaf) and the mean number in the third treatment was (26.7 pest/leaf).

These results agreement with those obtained by Heba (2013) in Egypt who reported that the plants *Zea mays* treatment with low concentration of triacontanol hormone TRIA (35 ppm.) was low infestation with *Euprepocnemis plorans plorans* (Charpentier) (Orthoptera. : Acrididae) comparing to control. The plants treated with high concentration of the same hormone (50 ppm.) were high infestation with the same insect comparing to control. Also, Gupta *et al.* (2009) reported the role of CKs in pest control and reported that plants treated with low concentration of CKs were less infestation by insects than control plants. Singh and Bhattacharya (2001) recorded an efficient role of CKs in reduction of survivorship and developmental parameters of larvae of *Spilarctia oblique* Walker

(Lepidoptera: Arctiidae) upon feeding on diets containing CKs, referring to insecticidal activity of CKs. From the entire last, it was suggested the incorporation of CKs in the Integrated Pest Management (IPM) modules for pest control.

3. Effect of cytokinin hormone (CKs) on the morphological characteristics and internal components of cucumber plants:

Table (3): Effect of treated cucumber plants by different concentrations of cytokinin hormone (CKs) on the morphological characteristics and internal components of these plants during 2018 season

Adjective	25 ppm	45 ppm	65 ppm	Control
Root length (cm)	110.76 ^c	97.32 ^a	82.42 ^b	95.25 ^a
Shoot length (cm)	155.43 ^c	140.25 ^a	125.28 ^a	135.21 ^a
Plant height (cm)	266.19 ^c	237.57 ^b	207.70 ^b	230.46 ^a
Total protein (mg/g)	19.75 ^c	16.58 ^a	11.25 ^a	15.47 ^a
Total sugars (mg/g)	32.84 ^c	27.35 ^a	21.46 ^b	25.73 ^a
Strach (mg/g)	45.65 ^c	37.46 ^a	30.78 ^b	35.86 ^a
Amino acids (mg/g)	15.13 ^c	10.63 ^b	6.67 ^a	9.75 ^a
Total phenol (mg/g)	13.65 ^c	8.64 ^a	4.35 ^b	7.86 ^a

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

Data showed that cucumber plants which treated with low concentration of CKs (25ppm) improved morphological characteristics of treated plants such as (root length, shoot length and plant height) and internal components of cucumber plants such as (total protein, total sugars, starch, amino acids and total phenols) compared to control, cucumber plants which treated with medium concentration of CKs (45ppm) were had no significant differences in both morphological characteristics and internal components of these plants compared to control, and cucumber plants which treated with high concentration of CKs had badly effect of the morphological characteristics and internal components of cucumber plants compared to control.

These results agreement with those obtained by Kumaravelu *et al.* (2000) in India who reported that the morphological characteristics such as (root length, shoot length, plant height and other morphological

Data tabulated in Table (3) show the effect of treated cucumber plants by different concentrations of cytokinin hormone (CKs) on the morphological characteristics and internal components of these plants.

characteristics) and physiological characteristics such as (total protein, total sugars, starch, total phenol and other physiological characteristics) were improved when the treated plants with small and medium concentrations of cytokinin hormone (CKs) and became better than control. These characteristics were worse than control when treated plants with high concentration of (CKs). Shukla *et al.* (2013) in Netherlands studied effect of cytokinin hormone (CKs) at lower concentrations on growth, plant hormones and artemisinin yield in *Artemisia annua* L. and found when treated plants with (CKs) produced a statistically significant positive effect on artemisinin level as well as on plant height, leaf and herbage yield, but these adjectives decreased when treated plants with higher concentrations of (CKs). Also these results agreements with those obtained by Eriksen *et al.* (2015) in Oslo (Nerweg) who reported that when treated tomato and maize plants with cytokinin

(CKs) caused a significant increase in the dry weight of the tomato plants, leaf area and dry weight measurements of tomato leaves at different stages of development. Richard and Stanley (1981) in Michigan (United States) reported that cytokinin (CKs) increased fresh and dry weight and total reducible nitrogen (total N) of rice (*Oryza sativa* L.) seedlings.

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