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Effect of *Cucumber mosaic virus* (CMV) on the Content of Some Cucumber Genotypes of Nitrogen, Protein, Phenols, and Flavonoids

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Abstract:

This study was carried out to secreen the response of ten cucumber genotypes (AH-38، HA-37،HA-2160 ،HA2114، HA-2122،HA-12 ،HA-16 ،HA-41 ،CU-O719 , and CU-2102) against *Cucumber mosaic virus* (CMV) and to know the effect of infection on some of the plant contents of nitrogen, protein, phenols, and flavonoids. This study was carried out in the laboratory of Plant Virology and plastic houses of the College of Agriculture, University of Kerbala. The results showed that all cucumber genotypes tested in this study are susceptibe to CMV with severity infection ranged from 15-100%. It was also proved that the viral infection had a clear effect on reducing the content of the plant of nitrogen and protein in the genotypes and the most affected genotype was HA-2122 that was a significantly different from the content of nitrogen and protein in the non-infected plants. CMV was also found to have an effect in increasing the plant content of phenols and flavonoids in all genotypes infected with the virus (CMV) and the most affected genotypes were HA-37 and HA-41 (2.51 and 2.42 mg g^{-1} dry weight, respectively) and significantly different from the content of the same non-infected genotypes that gave rates of 1.66, 1.78 and 1.71 mg g^{-1} dry weight, respectively.

Keywords: *Cucumber mosaic virus, Cucumber, nitrogen, protein, phenols, flavonoids.*

Introduction

The cucumber crop (*Cucummis sativus* L.) belonging to the family of *cucurbitaceae* is one of the vegetable important crops for its nutritional value, as it contains proportions of water, carbohydrates and protein and also contains some calories and a quantity of phosphorus, iron, vitamin B, niacin and ascorbic acid (Chakraborty et al., 2021). Cucumbers are consumed fresh or cooked as well as used in pickling, in addition to they have multiple medicinal uses (Sharma et al., 2020; Trak et al., 2022). The cucumber plants is infected with many viruses, including *Cucumber mosaic virus*, CMV, which is one of the most important and widespread viruses that may cause a loss of crop up to 80% (Li et al., 2020).

CMV has a wide family host, and infects more than 1,287 plant species belonging to 100 plant families (Mrkvová et al., 2022). The virus has different modes of transmission, including by the plant sap, seeds, and some insects such as

aphids such as *Myzus* persicae and Aphis gossypii, which are the most important species in the transmission of this virus (Sun et al., 2022). This study aimed to screen the response of some of cucumber gentypes against CMV and to find out the effect of the infection on the plant content of nitrogen and protein phenolic compounds and flavonoids.

Materials and Methods

Source of the CMV Isolate

An isolate of CMV prviously molecular dignosed was obtained from the Plant Virology Laboratory/ the college of Agriculture/ University of Karbala. This isolate of the virus was activated by making an extract from a plant sample infected with the virus using 0.07 M Sörenson phosphate buffer and mechanically inoculating young plants (3-5 true leaf stage). The control treatment was also carried out by inoculating other plants only with phosphate buffer solution. After 30 min of inoculation, all inoculated plants were washed with water. All plants were maintaned in a insect-free plastic house and monitered periodically to record any appearance and development of disease symptoms during the peroid of the experiment.

Preparation of Cucmber Gentypes Plants and Inoculation with CMV

Seeds of ten cucumber genotypes obtained from Green AL-Murooj Company/ Babylon Governorate were screened agaist CMV (Table 1). These genotypes were not previously known locally, and first tested in Iraq to find out their response to CMV by planting them separately in small pots containing sterile peatmos in the plastic house of the Department of Plant Protection/ College of Agriculture/ University of Kerbala. 15 days after planting, the plants were planted in the plastic house.

Table 1. Cucumber Genotypes This Study to Determine their Response Against CMV

Genetic makeup	Origin	Producing Company
AH-38	Holland	Gold seeds
HA-37	Holland	Gold seeds
HA-2160	Holland	Gold seeds
2114 HA-	Holland	Gold seeds
2122 HA-	Holland	Gold seeds
HA-12	Holland	Gold seeds
HA-16	Holland	Gold seeds
HA-41	Holland	Gold seeds
CU-O719	China	Beit alpha
CU-2102	China	Beit alpha

After the plant reached the age of 4-6 true leaves, all plants were mechanical inoculated with CMV. A control treatment was carried out by inoculating other plants of the same genotypes only by phosphate buffer solution. All plants were monitered daily for recording the appearance and development any symptoms until the end of the trial period. After 30 days of inoculation, all plants were tested by reverse polymerase chain reaction (RT-PCR). The severity of infection with the virus was also calculated according to the key previously described by Wang et al. (2011). Disease severity was measured using a 0-10point rating scale, according to Murphy et al. (2003): 0= no symptoms; 2= mild mosaic symptoms on leaves; 4= severe mosaic symptoms on leaves; 6= mosaic and deformation of leaves; 8= severe mosaic and deformation of leaves; 10= severe mosaic and deformation of leaves with stunted growth. The severity of the injury was calculated according to the equation below.

$$\text{Infection severity (\%)} = \frac{\text{Number of infected plants} \times \text{Degree}}{\text{Number of plants examined} \times \text{Highest score}} \quad (1)$$

Effect of CMV on the Content of the Fruits of Nitrogen and Protein

Nitrogen and protein analysis

The total nitrogen content of the fruits was measured using the Kjeldahl device by taking five ml from the previously digested sample and adding sodium hydroxide to it according to the following equation described by Al-Sahaf (1989).

$$Nitrogen = \frac{Q \times p \times R \times atomic\ weight\ of\ nitrogen \times 100}{A \times b \times 1000} \quad (2)$$

Whereas:

Q = the volume of acid used.

P = caliberation of the acid used in the process of qualiberation.

R = size of the digested sample.

A = the size of the diluted sample.

B = the weight of the plant sample.

Protein Analysis

The percentage of the protein was then calculated according to the following equation (Fisher and Hart, 1971).

$$Protein = nitrogen \times 6.25 \quad (3)$$

Effect of CM) on the Content of Phenols and Flavonoids

Phenolic analysis

Total phenols were analyzed according to the method adopted by Singleton (1974) and modified by Pérez-Gregorio (2021), one ml of ethanol fruit extract was taken and one ml of distilled water was added, and then five ml of Folin Ciocalteu reagent was added at a concentration of 10% (volume/volume). The sample was left for 10 minutes and then added to four ml of sodium carbonate (Na_2CO_3) at a concentration of 7.5%. The samples were then left for 90 min at a temperature of 25 ℃. The absorption readings of the samples were then taken at a wavelength of 765 nm.

Flavonoids Analysis

The method described by Zhishen et al. (1999) was used according to the steps described below:

1- Methanol alcohol (1.5ml) was added to 0.5ml of ethanoli fruit extract and mix well with the help of a vortex mixer.

2- One ml of aluminum chloride solution (AlCl3) was added at a concentration of 10%.

3- Potassium acetate (CH3CO2K) (0.1 ml) was added, distilled water added with a volume of 2.8 ml and then the sample was left at room temperature for 30 minutes.

4- The bsorption readings of the samples were taken at a wavelength of 510 nm.

Statistical Analyses

Descriptive statistics have used Frequency, percentage tables mean and standard deviation). F test was used to compare means between three or more independent groups. Level of significance of ≤ 0.05 was considered as significant difference. Least significant difference (LSD) was used to find differences between groups (Al-fahham, 2018).

Results and Discussion

Severity of CMV Infection in Cucumber Genotypes

results showed that all cucumber genotypes tested in this study were susceptible to CMV with those genotypes varying with each other at the time of appearance and severity of the resulting disease symptoms. The genotypes HA-37, HA2122 and HA41 were found to be the most sensitive to CMV among the other

genotypes, with a severity of infection of 80 and 100%, respectively. As for the genotypes HA-38-HA12-HA16 and CU-O2102, they were less sensitive to the virus and the severity of infection ranged from 40-60%, while the genotypes HA-2114, CU-O719 and HA-2160 recorded a lower sensitivity to the virus compared to other genotypes, with a severity of infection of 15-20%, respectively. The reason for the clear difference in the severity of infection is due to the genetic differences of the genotypes that play an important role in resistance or sensitivity to viral infection. These results in agreement with Al-Yasiri (2016).

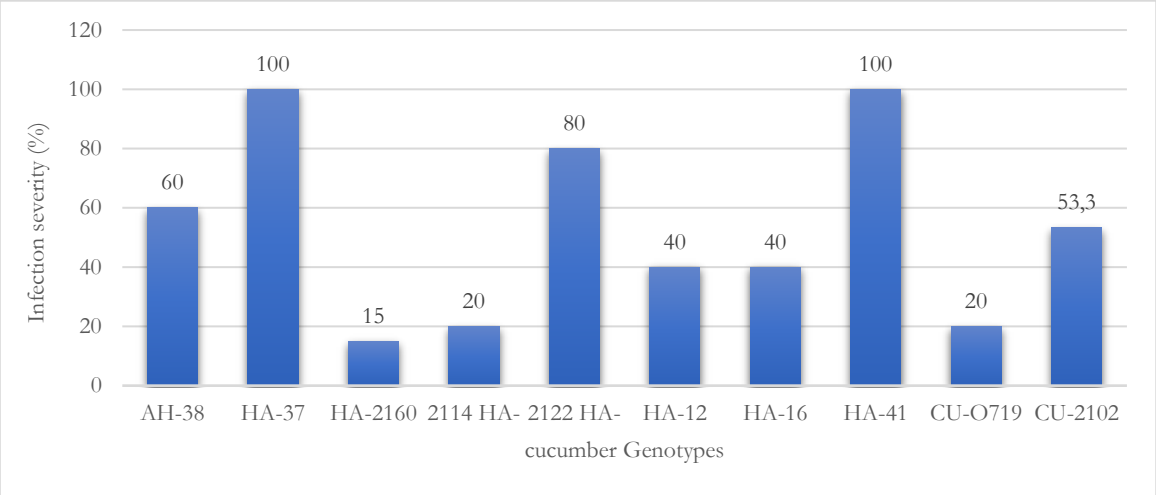

Figure 1. Severity of CMV Infection in the Genotypes of Cucumber Plants (AH-38, HA-37, HA-2160, HA2114, HA-2122, HA-12, HA-16, HA-41, CU-O719 and CU-2102) Tested in this Study

Effect of CMV Infection on the Content of Some Genotypes of Some Chemical Compounds and Total Nitrogen

The results showed that the nitrogen content in cucumber fruits was also affected by CMV (1.76%) that had a significant difference from the nitrogen content of uninfected plants, which amounted to 2.62%. The fruits of the genotypes also differed in their content of nitrogen. It was found that the highest rate of nitrogen was in the fruits of the genotype HA-12 (2.96%), while the fruits of the genotype HA-2122 had the lowest content of this element among the other genotypes, with a rate of 1.76%. As for the rest of the genotypes, they showed significant differences among them, with rates ranging between 2.96-1.76% (Table 2).

The interaction between the genotypes of infected and uninfected plants showed that the genotype HA-41 infected with the virus gave the lowest content of this element (1.13%) with a significant difference from the content of the fruits of the same uninfected genotype (2.56%), while it was found that the highest nitrogen content was in the genotype HA-2122 infected with the virus (2.81%), which differed significantly from the content of the fruits of the same uninfected genotype, which gave an average of 2.86%.

Table 2. Effect of CMV on the Nitrogen Content in the Fruits of Some Cucumber Genotypes

Genotype	Nitrogen		Mean
	Non-infected plants	Infected plants	
AH-38	3.50	1.84	**2.67**
HA-37	2.05	1.69	**1.87**
HA-2160	1.86	1.73	**1.79**

HA-2114	2.86	2.81	**2.33**
HA-2122	2.27	2.24	**1.76**
HA-12	3.59	2.33	**2.96**
HA-16	2.27	1.95	**2.11**
HA-41	2.56	1.13	**1.85**
CU-O719	2.98	2.19	**2.58**
CU-2102	2.25	1.76	**2.00**
Mean	**2.62**	**1.76**	
L.S.D $_{(0.05)}$	Genotypes	Infection	Interaction
	0.55	**0.24**	**0.77**

Protein

The data shown in Table (3) indicate that CMV infection led to a decrease in the protein content (11.05%) and it is significantly different from its presence in the non-infected plants, which amounted to 16.39%. The genotypes also showed a difference in their content of protein. The genotype HA-12 gave the highest content (18.50%), which differed significantly from the percentage of protein presence in the fruits of other genotypes, which was the lowest in the genotype HA-2122 (11.01%). It is also clear through the interaction between infected and uninfected genotypes that the HA-41 genotype infected with CMV had the lowest protein content (7.08%), which differed significantly from the content of the fruits of the same genotype non-infected with CMV (14.20%), while the fruits of the HA-12 genotype infected with CMV gave the highest protein content (14.56%) that was significantly different from the content of the fruits of the same genotype not infected with the virus.

Table 3. Effect of CMV on the Fruits content of Protein in Some Cucumber Genotypes

Genotype	Protein		Mean
	Non-infected plants	Infected plants	
AH-38	21.87	11.50	**16.68**
HA-37	12.81	10.60	**11.70**
HA-2160	11.66	10.81	**11.23**
HA-2114	17.91	11.31	**14.61**
HA-2122	14.23	7.79	**11.01**
HA-12	22.43	14.56	**18.50**
HA-16	14.20	12.18	**13.19**
HA-41	16.04	7.08	**11.56**
CU-O719	18.62	13.70	**16.16**
CU-2102	14.10	11.00	**12.55**
Mean	**16.39**	**11.05**	
L.S.D $_{(0.05)}$	Genotypes	Infection	Interaction
	3.44	**1.54**	**4.87**

The results proved the effect of CMV infection significantly on the plant contentof nitrogen and protein due to the effect of the virus on many biological and physiological processes in plants that led to a decrease in the plant efficiency in absorbing many mineral elements from the soil, including nitrogen, which significantly affected protein synthesis and low its concentration in the plant. Khudair et al. (1986) and El-Hammady et al. (1983) and Al-Barzanji (2005) indicated that infection with *Bean mosaic virus* (BYMV) led to a clear decrease in the plant nitrogen content and its negative impact on the of protein in plants. In a study conducted by García-Latorre (2021), it was proved that plants infected with some pathogens, including infection with *Fusarium* spp. decreased their content of nitrogen and protein. AL-Abedy et al. (2021) showed a significant decrease in the nitrogen and protein

content of okra plants infected with *Fusarium culmorum*.

Effect of CMV on Some Chemical Compounds in Cucumber Fruits

Phenols

It is evident from the data shown in Table (4) that the total phenols content of the fruits of the plant infected CMV increased at a rate of 2.05 mg g^{-1} dry weight compared to 1.51 mg g^{-1} dry weight in the fruits of plants not infected with the virus .It was also found that there was a difference between the genotypes in their content of phenols, which was the highest at the genotype HA-37 (2.51 mg g^{-1} dry weight) that was significantly different from the content of phenols in the fruits of the genotype HA-2160 (1.48 mg g^{-1} dry weight). It was also found that there is a clear difference between the other genotypes in their content of phenols with rates ranging between 1.48 - 2.51 mg g^{-1} dry weight).

Regarding the interaction, the genotype HA-37 infected with CMV gave the highest percentage of increase in the content of plant fruits of phenols, which was 2.62 mg g^{-1} dry weight that was significantly different from the same genotype not infected that was 2.40 mg g^{-1} dry weight. The genotype CU-2102 (1.56 mg g^{-1} dry weight) differed significantly from the content of the fruits of the same uninfected genotypes.

Table 4. Effect of CMV on the Phenols Content in Some Cucumber Genotypes

Genotype	Phenols		Mean
	Non-infected plants	Infected plants	
AH-38	1.19	2.13	1.66
HA-37	2.40	2.62	2.51
HA-2160	1.15	1.81	1.48
HA-2114	1.55	1.67	1.61
HA-2122	1.57	2.36	1.96
HA-12	1.39	1.68	1.53
HA-16	1.62	2.23	1.92
HA-41	1.29	2.44	1.86
CU-O719	1.69	2.02	1.85
CU2102	1.27	1.56	1.41
Mean	1.51	2.05	
L.S.D $_{(0.05)}$	Genotypes	Infection	Interaction
	0.50	0.22	0.71

Flavonoids

It was found that the presence of flavonoids was significantly affected in fruits of plants infected with CMV (2.28 mg g^{-1} dry weight) compared to the normal content recorded in the fruits of uninfected plants, which was 1.55 mg g^{-1} dry weight. It was also found that there was a difference in the level of flavonoids in the genotypes, which was the highest in the genotype HA-41 (2.52 mg g^{-1} dry weight) that significantly different from its presence in genotype CU-2102, which recorded an average of 1.60 mg g^{-1} wt. dry (Table 5).

It was also evident from the interaction between the infected and uninfected genotypes that the fruits of the infected genotype HA-41 had the highest content of flavonoids (3.15 mg g^{-1} dry weight) compared to the fruits of the same uninfected genotype (1.89 mg g^{-1} dry weight), while the genotype CU-2102 recorded the lowest presence of flavonoids (1.70 mg g^{-1} dry weight) compared to its concentration of 1.50 mg g-1 dry weight in plants not infected with the virus.

Table 5. Effect of CMV on the Flavonoids Content in Some Cucumber Genotypes

Genotype	Flavonoids		Mean
	Non-infected plants	Infected plants	
AH-38	1.42	2.14	1.78
HA-37	1.36	2.51	1.93
HA-2160	1.47	2.00	1.74
2114 HA-	1.53	2.30	1.92
2122 HA-	1.33	2.74	2.04
HA-12	1.88	2.04	1.96
HA-16	1.73	2.32	2.02
HA-41	1.89	3.15	2.52
CUO719	1.38	1.89	1.63
CU-2102	1.50	1.70	1.60
Mean	1.55	2.28	
L.S.D $_{(0.05)}$	Genotypes	Infection	Interaction
	0.51	0.23	0.73

Conclusions

Scalbert (1991) indicated that the increased production of phenols, flavonoids in the plant leads to an increase in plant resistance to pathogens, as phenols and flavonoids inhibit the development of diseases by inhibiting enzymes such as Laccase, Pectinases, Cellulases, and Xylanase that are produced by pathogens outside cells that have a role in its pathogenicity, as well as its role in inhibiting the process of oxidative phosphorylation in fungi and deprive them of minerals, proteins and antioxidants in plant tissues. Agrios (1997) also indicated that some compounds such as phenols and flavonoids, whether they are present in the plant or formed after infection that have an effective role in plant resistance against pathogens. These results also agreed with what Al-Shami et al. (2018) indicated that viral infection in plants increases the formation of phenolic compounds inside the plant as reactions against the virus. Qi et al. (2016) noticed that the concentration of these compounds in plants infected with *Fusarium oxysporum* was higher than in the non-infected plants. Ramakrishna et al. (2011) stated that the deficiency of nitrogen, potassium and magnesium in plants leads to an increase in the production of flavonoids in different plant systems, and this was demonstrated by the results of the current study about the decrease in the above nutrients due to infection with CMV and a significant reduction in the content of plants of nitrogen and potassium, which led to an increase in the presence of flavonoids in the affected plants.

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