Efficiency of algae Sprulina platensis the and the aqueous extract of Moringa oleifera seeds and leaves to induce ten cultivars of cucumber plants against the Cucumovirus Cucumber Mosaic Virus (CMV)

Basma Dhabab Al-ajeele (1) **Maadh Abd Al- Wahab Al Fahad(2)**
1*Plant protection, collage Agriculture. Tikrit Uni. Email: basma2020@tu.edu.iq
2*Plant protection, collage Agriculture. Tikrit Uni. Email: maadh.alfahd@tu.edu.iq

Abstract:
This study is aimed to identify the effect of the locally grown algae Spirulina platensis in the form of an biological in vitro preparation on Cucumber Mosaic Virus (CMV) and the effect of Moringa leaf extract M. oleifera by evaluating some resistance parameters, which included measuring the infection ratio, its severity, the activity of the peroxidase enzyme, and the amount of phenols. This study was conducted in the research station of the College of Agriculture / University of Tikrit. CMV was initially diagnosed using immune strip assay, and the diagnosis was further enhanced by using Next Generation Sequence (NGS) modern technology to detect the complete genome of the virus strain .
The infection rate was 100% in the control treatment, While lower infection rate for both treatments of algae S. platensis and M. oleifera, was 31% for each of them. As for the effect of the two treatments on the severity of infection, it was the lowest in the Ghazeer cultivar for both treatments of algae S. platensis and M. oleifera, reaching 40% compared with others of the cultivars which differed significantly, The highest infection rate 80% was recorded in the two treatments of S. platensis and M. oleifera for Jade cultivar compared with control reached 100%.
The results of the peroxidase enzyme measurement showed the highest value for the Ghazeer variety for the two treatments used S. platensis and the M.oleifera which amounted to 3.1 units/mg protein of the root system and for the two treatments used which are the algae S. platensis and the M. oleifera. The rate of total phenols increased to 8.9 mg/g wet weight in treatment S. platensis for the Ghazeer variety while M.oleifera reached 7.9 mg/g wet weight compared the control treatment witch reached 4.6 mg/g wet weight.
The results of the varieties used in this study showed a significant difference and the variety the Ghazeer showed superiority in tolerating infection, While the Jade variety was the most sensitive variety to infection CMV. The early treatment before the infection occurred were superior in all studied traits to the treatment conducted after the infection.
Key words: Sprulina platensis, Moringa oleifera, Cucumber Mosaic Virus (CMV), ISR, NGS

Introduction

The cucumber plant (Cucumber sativus L.) is one of the oldest vegetable crops that cultivated by man, and it belongs to the cucurbit family. It comes in the second place after the tomato for its nutritional value [1]. The cucumber is grown throughout the year in Iraq using of the open and covered pattern, so it is exposed to attack by Various types of viral, bacterial, fungal, phytoplasmic and nematode pathogens as a result of the increase in cultivated areas [2], and the most important one of these Pathogen is Cucumber mosaic virus (CMV) [3] and because the unuseful use of pesticides with viruses diseases, so the research has turned towards strategies for managing the disease [4] the thinking has tended to activate these mechanisms, and to treat the plant with biological factors Such as extracts, compounds and biological factors that can activate these mechanisms and stimulate systemic resistance against pathogens [4] and .[5]

Algae belonging to the genus S. platensis have been used in the biological control of several plant pathogens [6],[7], [8], [9], [10], [11], [12]. In addition to other nutritional uses for humans or as animal feed, S. platensis has proven to control many pathogens and viruses diseases [13] due to its high protein content,

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up To 60-70% and an increase in the concentration of amino acids, antioxidants, minerals and vitamins [14], as well as its content of alkaloids and cyclic peptides that contribute to reducing viral infection [15], [16], S. platensis increase The inhibitory ability to multiply viruses by raising the plant's ability to biosynthesize amino acids, which positively affects compensation for the imbalance of nitrogenous bases as a result of viruses infection, and this gives less opportunity for the virus to express itself within the infected plant cell and thus reduce the rate and severity of infection [17], [18 ,[.[13]

The M. oleifera plant is considered as a one of the medicinal plants that belong to the Moringaceae family and are used to treat many diseases as a medicine [19] Researchers turned their attention to the M. oleifera plant and its seeds because it has microbial activity against pathogens, as it is characterized by its containing effective plant components, including alkaloids, flavonoids, and sugars Reductants, steroids, carbohydrates, cyanoins and tannins [20] In a recent study, two effective compounds were isolated from moringa seeds and known for their antiviral activities [21]. It was also shown in a laboratory study that the activity of moringa extract against phytopathogenic fungi reduced the radial growth of fungi significantly even when low concentrations.[22]

Due to the importance of the crop and the wide spread of the virus, the study tended to develop an integrated strategy in reducing the impact of the pathogenic virus and the absence of local studies related to the Moringa extract M. oleifera. This study aimed to:

(1Growing S. platensis algae in the laboratory under appropriate conditions for development and studying the effect of using it as a bio-resistant agent in a ready-made form and grown against CMV virus; 2) Studying the effect of M. oleifera extract in reducing some parameters of infection with CMV virus; 3) Evaluation of the response of some cucumber cultivars to infection based on resistance indicators; 4) Evaluation of the dates of adding treatment in the resistance to the CMV virus.

materials and methods.

Collecting the infected plant samples for diagnostic purposes.

Samples of cucumber plants showing pathological symptoms suggesting that they were infected with CMV virus were taken from the fields of Baiji District / Salah Al-Din Governorate and the greenhouses station in the College of Agriculture / University of Tikrit.

Serological diagnosis of CMV virus.

The Immuno strip assay kit was obtained from the American company Agdia biofords and equipped in the form of flash kits. The diagnosis was made for the infected samples in the Virus Research Laboratory of the Plant Protection Department - College of Agriculture - University of Tikrit and according to the program developed by the equipped company according to Next steps:

1)Take 0.15 gm. of plant tissue showing symptoms of infection, cut a sample of the tissue using a sharp sterile knife.

(2The sample was crushed well to remove the vegetable juice from the sample, and its environment was modified with Buffer solution and left for three minutes.

(3The end of the flash kits was immersed in the bag containing the sample solution to be tested for a distance of 0.5 cm.

4)The strip was left in the extract for 30 minutes for the reaction to occur and the result to be monitored, by the appearance of a sedimentation line on the immune strip assay or if it did not appear in the absence of the CMV virus in the sample. The method was also conducted with healthy plants for comparison.

Molecular Diagnosis of CMV Virus.

The virus was diagnosed using Next Generation sequencing technology, and the RNA sequence was read according to the specifications approved by DNA Link Inc. in South Korea, using the Novaseq6000 program. 101 Baired End According to the company's Trueseq RNA method, this sequence was checked by Blast software.

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Preparing the treatments used in inducing virus resistance.

The experiment included conducting treatments on ten varieties of cucumber plants, and each treatment was repeated according to the dates and for all varieties.

1. Treatment of the algae Spirulina platensis, symbolized by S. platensis, and infected with CMV virus.
2. Treatment of Moringa oleifera and its symbol is M. Oleifera and infected with CMV virus
3. Control treatment infected with CMV virus only.

The source of the treatments and the way they are used.

1-Preparation of S. platensis isolate for bio-mass production.

The diagnosed isolated of S. platensis was obtained from the laboratory of the Department of Food Sciences - College of Agriculture - University of Tikrit, which was previously brought from the National Research Center in the Arab Republic of Egypt, and it was diagnosed and confirmed for its purity based on the taxonomic source [23] as shown in the figure (2-1), the materials needed for the saline development medium were prepared accurately for the purpose of development and propagation in a prepared form in the Virus Research Laboratory - College of Agriculture - Tikrit University.

The figure (2-1): A+B= Growth stages of algae *S. platensis* in the virology laboratory

Preparation of *S.platensis* algae propagation media

The media for alga propagation (S. platensis) was prepared according to the method of Zarrouk (1966) by dissolving several types of salts mentioned in Table (1-3) in one liter of distilled water. They precipitate with heat. As for the trace element solutions A and B, a liter of each was prepared in the form of a storable solution and kept in the refrigerator until use. 1 ml of each of the trace element solutions A and B was added to a liter of Zarok media. Then, the sterilization was carried out using an autoclave at 121˚C and a pressure of 1.04 kg/cm3 for 15 minutes.

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Table (2-2) Materials used in preparing Zarrouk salt media.

The weight g/L	Chemical symbol	Solution A+B+C = Zarrouk media
0.64	EDTA	**Solution A**
0.08	FeSO4.7H2O	
0.32	CaCl2	
1.6	MgSO4.7H2O	
8	NaCl2	
8	K2SO4	
2	NaNO3	
4	K2HPO4	
134.4	HGNaHCO3	
0.015	MoO3	**Solution B**
0.079	CuSO4.5H2O	
0.222	ZnSO4.7H2O	
1.81	MnCl2.4H2O	
2.86	H3BO3	
43.98	Co(NO3)2.6H2O	**Solution C**
61.10	TioSO4.H2SO4.8	
17.94	H2O	
44.80	Na2WO4.2H2O	
192.0	NiSO4.6H2O	
22.96	Kcr(SO4)3.12H2O	
	NH4NO3	

Preparation of *M. oleifera* seed and leaf extract

The samples were obtained from Moringa trees located in the nursery of the College of Agriculture / University of Tikrit, and followed the method used by [24] in the extraction process, as the dry plant parts were ground using a mill to obtain powder, and soaked in distilled water to obtain the aqueous extract, as 100 ml of water was added for every 10 gm. of dry powder or leaf powder, the mixture was left on a hot plate at 30 °C for 15 minutes, then left in the dark for 24 hours, after which the infusion was filtered using layers of medical gauze, after that filter paper was used, the filtrate was subjected to heteroptation vigorously 3000 r/min for 10 minutes in a centrifuge, then it was lyophilized in a lypholizer until the extract was dry, then the dry extract was scraped off with a clean and sterile knife and the dry powder was kept after weighing in clean and airtight plastic containers until use. This preparation was called the dry aqueous extract.

Treatment method.

1) **Treatment algae *S. platensis***:

The treated to The previously selected plants were done according to the scheme of the experiment with different dates as follows.

A) Before making infection: as the treatment was added to the two-week-old plants at a rate of 10 g/L, then they were infected at the age of 20 days by mechanical inoculation, and after a week of infection, they were treated with *S. platensis* a second and third time at the same concentrations.

B) After the infection: the seedlings were mechanically infected at the age of 20 days with the CMV virus, and after a week of infection they were treated with algae *S.platensis* for the first time, then again after a week, with the same concentrations.

2) **Treatment with *M. oleifera* extract**

The plants were treated with different dates as follows

A- Before the infestation: As the treatment was added to the two-week-old plants at a rate of 10 g/L, then they were infected at the age of

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20 days by means of mechanical inoculation, and after a week of infection they were treated with *M. oleifera* extract a second and third time at the same concentrations.

B- After the infection procedure: the seedlings were mechanically infected at the age of 20 days with CMV virus, and after a week of infection they were treated with *M.Oleifera* extract for the first time and then again after a week with the same concentrations.

3) Control treatment: It was infected with CMV virus and left without adding any treatment while protecting it from the arrival of viral vectors.

A- Before infection: the plants first, then inoculated with CMV virus, then the treatment was repeated weekly until harvesting.

B_ After infection: vaccination with CMV virus first, then she was treated weekly until harvesting.

Prepare the plants for the experiment treatments.

The experiment was carried out during the agricultural season of the spring on 1/4/2022 in Salah al-Din Governorate / Baiji district. The seeds of ten cultivars of cucumber plants were sown, and plastic dishes were prepared and filled with a sterile dry soil mixture and peatalgae at a ratio of (1:3) The experiment was conducted in a random design. The complete RCBD, as the experiment was divided into 30 sections, each sector containing 18 experimental units treated with algae *S. platensis* and extract of seeds and leaves of *M. oleifera,* and the control treatment protected with a Almalmal fabric, and after germination, the treatments and mechanical infection were carried out in the manner described in paragraph (5-2).

studied traits:

1) The percentage of virus infection: CMV was calculated according to the following equation: Infection percentage % $= \frac{\text{number of infected plants}}{\text{otal number of plants}}$ x 100
2) Severity of infection with CMV virus: a pathological index for the severity of infection with CMV virus defined by Prof. Dr. Maadh Abdel-Wahab, depending on the appearance of symptoms and their severity (Figure 2-3), and the severity of the infection of plants was measured by calculating the number of infected plants and the development of the degree of each plant, after that applied according to the following equation [25].

Severity of infection=

$$\frac{(Number\ plant\ infection\ \times 1) + (Number\ plant\ infection) \times 2) \ldots . \\ +(Number\ plant\ infection\ \times 6)}{The\ number\ of\ taken\ sample\ 5 \times (higher\ class)\ 6}$$

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The number	Picture	Describe symptoms
0		Healthy plant
1		yellowing on new leaves
2		Yellowing with roll leaves
3		Mosaic on leaves
4		Severe mosaic with yellowing of leaves
5		Vein clearing, Yellowing most leaves
6		Plant stunting, leaves severe deformed, and other symptoms above

Figure 2-3: Pathological evidence of the severity of infection with cucumber mosaic virus

3)- Measure the activity of the peroxidase enzyme (unit/mg protein).

The method was adopted to test the enzyme activity and the following equation was applied

Enzyme specific activity (mg/protein)

$$= \frac{enzyme\ activity}{protien\ concetration}$$

4)- The effect of infection with CMV virus on the concentration of total phenols mg/g wet weight of the root system.

The Total phenols were estimated according to the method [26] cut 1 gm from each of the root and vegetative system, then the samples were placed in test tubes of 25 ml capacity, and added 10 ml of ethyl alcohol at a concentration of 80% to each sample, then the samples entered in a boiling water bath For ten minutes, after that it took out and left it to cool, then crushed each sample using a ceramic mortar, and after completing crushing process, filtered the samples through two pieces of gauze, then repeated the process by adding another 15 ml of ethyl alcohol in order to complete the extraction process completely. The samples were crushed and filtered again, and the volume was completed to 25 ml, then take 1 ml of Arno's solution, 10 ml of distilled water, and 2 ml of NaOH solution at a concentration of 4% with the preparation of a buffer that includes all the above solutions except for the alcoholic extract, then the absorbance of the reaction mixture was measured using a UV-spectrophotometer at wavelength It is estimated at 515 nm to extract the concentration of total phenols.

Results and Discussion

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The Diagnosis by Immune strip assay for CMV virus.

The results of the examination with the immune strips assay in the picture (3-1) for the CMV virus showed a positive test, which indicates the presence of the virus in the cucumber plant, that showed symptoms of mosaic, deformation and reduction in size. As the sedimentation line appeared in a pink color containing the anti-CMV virus serum, this method was adopted in the diagnosis of viral infections [27].

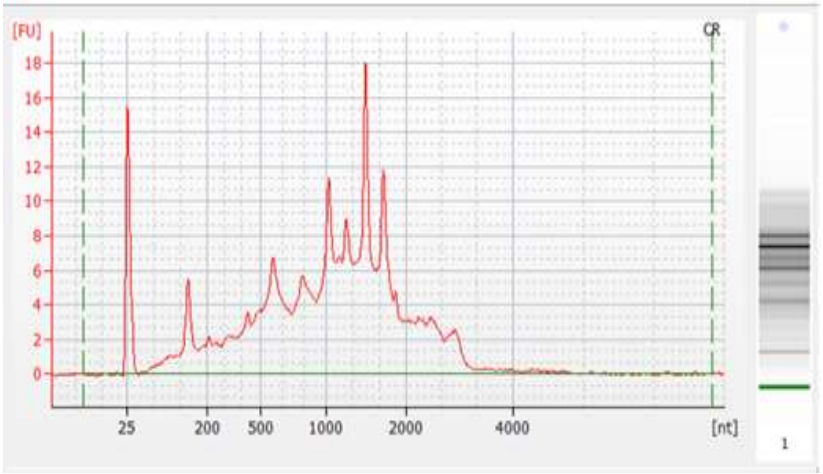
The picture (3-1) Positive reaction result of the plant extract infected with the CMV virus with the anti-CMV serum

Diagnosis by next generation technology of the RNA of the infected plant.

Extracting the RNA of the infected plant.

The results of RNA extraction from infected cucumber plants showed that the purity of the nucleic acid was 2.40 ng/µl. and a concentration of 104 ng/µl. The result of gel electrophoresis showed the presence of eight clear bands on the gel and the presence of eight curves in the Bioanalyzer device, which represent the types of RNA, the Figure (3-2)

The optical spectrum reading for the purity and concentration was between 1.8 to 2 and it is similar to the mentioned readings and many sources [28], [29], [30].



(3-2) The curves produced by the Bioanalyzer device show eight curves of the extracted RNA types on the left, as well as eight curves of RNA bands on the agarose gel on the right, sent by the diagnostic company

the Effect of treatments used on some resistance and growth indicators on cucumber plants infected with CMV virus.

the Effect of *S. platensis* and *M. oleifera* extract treatment on the percentage of the

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infection with CMV virus on ten cultivars of cucumber plants.

The statistical results of Table(3-1)showed a significant superiority of the alga treatment *S. platensis* and Moringa extract *M.oleifera* in reducing the percentage of infection with the virus, as it gave a percentage of31% for both, compared to the control treatment, which was 100%.

The cultivars Ghazeer and Oreka f1 showed the lowest infection rate of 31% with a significant difference compared to the two cultivars Jade and victoria, which did not differ significantly between them and recorded an infection rate of 75% for both. This is explained from the release of resistant cultivars by some genetic expressions in several Sites on the gene, that weakens virus transmission and hinders its access to healthy cells [31], and the difference in the ability of varieties to tolerate infection may be due to the effect of the treatment of the algae *S. platensis* for many reasons, including the production of compounds with an antibiotic effect, that support the production of natural phenols such as tannins. These substances They act as anti-diseases, and work to increase cell wall cell wall, which increases the plant's ability to resist diseases. It supports the production of enzymes responsible for the formation of phytoxins and inducing plant resistance (ISR) upon infection, or as a result of *S. platensis* containing cytokinins, which increases the rate of synthesis. The biosynthesis of mRNA, RNA, and DNA amino acids has a positive effect in compensating for the imbalance of nitrogenous bases as a result of viral infection, which gives less opportunity for the virus to express itself within the infected plant cell [32], [17].

It is possible to deduce the reason for reducing the infection in the treatment of *M. oleifera*. The extract has antibacterial properties, as it contains lipophilic compounds. These compounds can stick to the protein envelope of the virus and thus hinder its transmission within plant cells and its multiplication. It also contains antibiotic metabolism such as carboxylic acid and enzymes. Cell wall lysates [33].

Table (3-3) effect treatment on the percentage of the infection with CMV virus

variety	spirulina		Moringa		Control
	before	after	before	after	
Habib	43	50	50	50	91
Faiz	31	50	37	50	81
punjabi	62	75	66	66	100
Fried	37	50	43	50	82
Maten seed	42	50	50	62	91
Oreka f1	31	37	31	37	91
Beta alpha	50	62	50	66	91
Ghazeer	31	37	31	37	75
Jade	66	75	75	75	100
vectoria	66	66	66	75	100

the Effect of *S. platensis* treatment and *M. oleifera* extract on the percentage of infection severity with CMV virus in ten cultivars of cucumber plants.

The data in the Table (3-4) indicate that the *S. platensi* treatment and *M. oleifera* extract recorded the lowest infection severity, which amounted to 40% compared to the control treatment, which amounted to 100%.

The effect of cultivars differed, as the Ghazeer cultivar recorded the lowest rate of infection severity, amounting to 40% compared with other cultivars, that differed significantly and recorded the highest rate of infection severity,

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reaching 75% for both treatments compared to the control treatment, which amounted to 100%.

The reason for the lack of difference between the two treatments for the severity of the infection is due to the occurrence of systemic resistance stimulation in the plant, which makes it effectively resistant to viral infection until the end of its life cycle, and this is consistent with what was indicated by [2]. What confirms this is the increase in the effectiveness of the peroxidase enzyme and the amount of phenols, which will be explained in the coming paragraphs.

the study indicate a result that the method of addition led to a significant increase in the two treatments. Either the superiority of Ghazeer cultivar over the rest of the cultivars may be due to its containing more than one virus resistance gene or its ability to supply the vegetation system with enough materials, including sugars, necessary to reduce the negative effects of the virus and thus limit the apparent symptoms. On the plant and this came in agreed with [34]

Table (3-4) effect treatment on the percentage of severity the infection with CMV virus

variety	spirulina		Moringa		Control
	before	after	before	after	
Habib	56	66	56	66	94
Faiz	58	60	58	66	82
punjabi	72	78	72	78	100
Fried	48	55	48	58	86
Maten seed	50	55	52	58	86
Oreka f1	44	48	44	48	86
Beta alpha	60	62	62	66	94
Ghazeer	40	44	42	48	86
Jade	75	75	75	80	100
vectoria	72	75	72	80	100

the Effect of treatment of *S. platensis* algae and *Moringa oleifera* extract on the specific activity of peroxidase enzyme (unit/mg protein) on ten cultivars of uninfected cucumber plants infected with CMV virus:

The results that listed in Table (3-5) showed the effect of the treatments and cultivars of the infected plants on the enzyme activity of the root system. The cultivar Ghazeer recorded the highest enzymatic activity in both treatments of *S. platensis* and *M. oleifera* extract, which amounted to 3.1 units/mg protein and 3.0 units/mg protein compared With the control treatment, which amounted to 1.3 units/mg protein, followed by Faiz for both treatments with 3.0 and 2.7 units/mg protein, respectively, of the root system, compared to the control treatment, which amounted to 2.1 units/mg protein of the rootstock. The lowest value was for Jade cultivar, which reached 0.7 and 0.9 units/mg protein in both treatments of algae *S. platensis* and *M. oleifera* extract, respectively, compared to the control treatment, which was 0.4 units/mg protein of the root system.

The peroxidase enzyme is one of the defense proteins against diseases, and its presence increases with disease infection [35], [36] and it is an important indicator of plant infection with pathogens [37]. As it enters the building of the walls and gives them strength through the addition of lignin and the oxidation of phenols that increase the durability of the cellular wall and prevent penetration by the pathogen, so it reduces the movement of the virus inside the plant.

Algae contain natural growth regulators similar to auxins and cytokinins, such as indole acetic acid and indole butyric acid, which stimulate plant growth and prevent

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yellowing, and thus positively affect the building of the protein from which the peroxidase enzyme is produced.

The effect of the *M. oleifera* treatment extract is due to the active groups it possesses of alkaloids, amino acids, carbohydrates and phenols, which formed complexes with it, thus preventing it from binding to the sites of enzymes and inhibiting them [38] .

Some cultivars respond more than others, and a noticeable increase appears as a result of genetic factors related to the cultivar, which increases the enzyme rate and thus breaks down the auxiliary materials for viral infection. This is consistent with the results of [6], [11] .

The table (3-5) Peroxidase

variety	spirulina		Moringa		Control
	before	after	before	after	
Habib	1.50	1.10	1.40	1.20	0.90
Faiz	3.00	2.60	2.80	2.70	1.00
punjabi	1.20	0.60	0.90	0.80	0.50
Fried	2.80	2.10	2.30	1.70	1.10
Maten seed	1.70	1.30	1.80	1.50	0.70
Oreka f1	2.60	2.50	2.40	2.30	1.00
Beta alpha	1.80	1.50	1.50	1.20	0.60
Ghazeer	3.10	2.50	3.00	2.30	1.30
Jade	0.70	0.60	0.90	0.50	0.40
vectoria	1.00	0.80	0.70	0.60	0.50

the treatment effect of alga S. platensis and Moringa oleifera extract on the poly phenols for ten uninfected and infected cucumber plants with CMV virus :

Table (3-6) show the concentration of phenols in the S. platensis treatment, reaching 8.9 mg/g wet weight of the root system, while the extract was treated with M. oleifera 7.9 mg/g wet weight of the root system, compared to the control treatment, which amounted to 5.2 mg/g wet weight. The cultivar Ghazeer and Oreka f1 recorded the highest rate of activity for phenols, outperforming all cultivars.

It is noted that both treatments stimulated resistance in cucumber plants, and one of the methods of resistance is to increase the production rate of phenols in the treatment before infection compared to the treatment after infection, and that increasing the plant content of phenols stimulates the production of phytoxins, which inhibits the growth of pathogens, and it is known that phytoxins are formed in the plant after infection, it uses as a defensive tool, and it is one of the characteristics of resistant plants [39] The superiority of the cultivar Ghazeer over other cultivars can be explained by the fact that it has genes that were induced as a

result of the treatment with algae or helped to produce inhibitors or reducers to multiply the virus and support the growth of the cultivar, in addition to adapting the cultivar to environmental conditions or The type of soil that was using in cultivated, which shows a significant increase in the rate of phenols, and this was indicated by [15 .[32] , [

This increase is due to the absorption of elements from Sprulina, which ensures that the plant's need for mineral elements is met, thus increasing vital activities and preventing infection with pathogens.[40]

The M.Olifera treatment was superior. It is possible that the reason is that the seeds, leaves and fruits contain effective chemical compounds such as flavonoids, which are phenolic compounds that play a role in

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reducing the incidence of diseases. The antioxidant activity also occurs due to the presence of phenols that contribute to the formation of free radicals and oxidants and the inhibition of antioxidant enzymes, and this is agreed. With **.[41]**

Table (3-6) effect treatment on poly phenols

variety	spirulina befor	spirulina after	Moringa befor	Moringa after	control virus
Habib	5.53 k-r	5.3 l-t	5.06 o-v	4.66 r-y	3.03 c-f
Faiz	7.5 c-e	6.1 h-l	6.8 e-i	5.9 j-p	4.00 x-b
punjabi	4.16w-a	2.9 c-g	3.46 z-c	2.86 c-g	1.8 i-n
Fried	7.1 e-g	6.9 e-h	6.6 f-j	6.1 i-m	4.13w-b
Maten seed	6.6 f-j	5.53 k-r	6 i-n	5.3 l-t	4.13w-b
Oreka f1	8.6 ab	8.2 a-c	6.9 e-h	6.3g-k	4.13w-b
Beta alpha	4.5 t-y	4.23 v-z	4.06 x-b	4.03 x-b	2.2 f-l
Ghazeer	8.9 a	8.3 a-c	7.9b-d	7.2 d-f	4.66 r-y
Jade	2.8 c-g	2.5 e-j	2.53 d-i	2.06 i-m	1.26 mn
victoria	3.03 c-f	2.16 f-m	2.26 f-k	1.76 i-n	1.3 l-n

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