Chapter

The Activity of New Bio-Agent to Control *Cucumovirus Cucumber Mosaic Virus* (CMV)

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Abstract

CMV virus is worldwide, especially in temperate regions, where it can infect more than 800 plant species belonging to about 40 families. Although the main factor that the plant takes in order not to be infected is because it has preventive means that inhibit the direction of pathogens so that the infection occurs under conditions that suit it and suit its success. Cucumber Mosaic Virus belongs to the group of plant viruses to the genus Cucumovirus, as the virus particles are symmetrically spherical, not enveloped, with a diameter of 29 nm, and the virus has several strains that differ among themselves in terms of factors, symptoms of infection and methods of transmission. The stimulation of induced systemic resistance (ISR) leads to the interest of many researchers. Many types of research and studies have been conducted in the field of biochemical changes in the form of modulating the host's cell wall. The production of phytoalexin. And the manufacture of pathogen-related proteins (Pathogenesis Related Protein). It has been indicated that treatment with various factors, for example (non-pathogenic organisms, weak pathogens, chemical and industrial compounds, plant extracts, nutritional supplements) has the ability to activate plant defense mechanisms and induce systemic resistance against pathogens. In the field of biological control, bacterial types have been used on many pathogens, including fluorescens Pseudomonas and Bacillus subtillus, as they have proven effective in controlling many different fungal and bacterial pathogens as well as viral, and the reason is due to the ability of the bacteria to produce many growth regulators and thus stimulate resistance The systemic plant and the production of phytotoxins are in addition to being one of the most important growth stimuli. New methods have been used to resist viruses by using natural nutritional supplements with effective effect, because plants have defensive means, and for this reason, the use of these supplements can be stimulated in addition to the preventive aspect, a decrease in infection parameters, and an increase in growth indicators and outcome. Several methods have been relied upon to diagnose viruses, the first being the symptoms of reagents, and they are of basic methods. After that, serological tests were adopted, which are highly specialized and accurate in diagnosing viruses, and electron microscopy was used as a method to detect the size and shape of viruses. Polymerase Chain Reaction (PCR) technology is a fast and accurate way to detect plant viruses compared to other tests, such as the ELISA test and plant reagents.

Keywords: biological control, cucumber mosaic virus, spirulina, food supplements

1. Introduction

Plant diseases are one of the main problems facing agricultural production worldwide, as they cause great losses to basic food crops. They may also lead to a decline in agricultural production. It is one of the plants belonging to the family Cucurbitaceae, and it is one of the summer vegetable crops grown in Iraq and that it is available throughout the year through the use of the open and covered agricultural pattern, according to the conditions that suit production [1].

CMV virus is worldwide, especially in temperate regions [2], where it can infect more than 800 plant species [3] belonging to about 40 families [2]. Although the main factor that the plant takes in order not to be infected is because it has preventive means that inhibit the direction of pathogens so that the infection occurs under conditions that suit it and suit its success.

CMV belongs to the group of plant viruses to the genus Cucumovirus, as the virus particles are symmetrically spherical, not enveloped, with a diameter of 29 nm, and the virus has several strains that differ among themselves in terms of factors, symptoms of infection and methods of transmission [4].

The stimulation of induced systemic resistance (ISR) leads to the interest of many researchers. Many researches and studies have been conducted in the field of biochemical changes in the form of modulating the host's cell wall [5]. The production of phytoalexin [6]. And the manufacture of pathogen-related proteins (Pathogenesis Related Protein) [7]. It has been indicated that treatment with various factors, for example (non-pathogenic organisms, weak pathogens, chemical and industrial compounds, plant extracts, nutritional supplements) has the ability to activate plant defense mechanisms and induce systemic resistance against pathogens [8].

In the field of biological control, bacterial types have been used on many pathogens, including *Pseudomonas fluorescens* and *Bacillus subtillus*, as they have proven effective in controlling many different pathogens, and the reason is due to the ability of the bacteria to produce many growth regulators and thus stimulate resistance in plant and the production of phytotoxins are in addition to being one of the most important growth stimuli.

New methods have been used to resist viruses by using natural nutritional supplements with effective effect, because plants have defensive means, and for this reason, the use of these supplements can be stimulated in addition to the preventive aspect, a decrease in infection parameters, and an increase in growth indicators and outcome [9].

Several methods have been relied upon to diagnose viruses, the first being the symptoms of reagents, and they are of basic methods [10].

In this chapter of the book we will discuss the following topics:

1. Isolation and molecular diagnosis of local isolation of CMV virus by RT-PCR assay.

2. To test the sensitivity of three cucumber genotypes against CMV.

3. Evaluation of the natural food supplement produced from *Spirulina sp* against CMV.

4. Evaluating the effect of *P. fluorescens* inoculation on infection rate and severity of CMV infection and some growth parameters and yield of cucumber varieties against cucumber mosaic virus.

5. Evaluation of the biological efficacy of medicinal plant extracts (licorice, sespan, and albizia) in inhibiting CMV infection.

6. The effect of the complementary treatment with the treatments mentioned above on the seeds on the percentage of their transmission and germination strength.

7. Determining the rate of transmission of the virus in seeds from different sources (local and international).

2. Review of literatures

2.1 Cucumber mosaic virus

2.1.1 Symptoms

The pathological symptoms of *Cucumovirus Cucumber Mosaic Virus* are described, which are characterized by the yellowing and mottling that spreads on cucumber leaves and also on the fruits (**Figure 1**), which is a yellow mottling that affects all leaves and after infection the leaves are deformed in addition to stunting, which is accompanied by gradual plant degradation and curling of the leaves, as well as surface roughness. The outer layer of the leaf and the appearance of small prominent growths, these growths are clearly between the small veins [11]. Mosaic cucumber is from the Cucumovirus group that works on the appearance of mosaics and mottles on their families. Gibbs and Harrison [12] stated that this virus causes green mosaic or alternating green and yellow colors with each other on cucumbers.

The virus causes symptoms of bundling veins and deformation of the leaves, in addition to the appearance of mosaic and the curvature of modern leaves, and the necks of the leaves are reduced and accompanied by deformation of the fruits on

FIgure 1.
CMV pathological symptoms.

the pepper plant [13], but on the mosaic eggplant and dwarfism, it is clear, and necrosis rings sometimes appear on the leaves, general distortion and layout [14].

The virus generally causes clear symptoms of mosaic on the leaves and many fruit trees and other plants, in addition to general stunting of banana trees, ornamental plants, and gladiolus, where it produces symptoms similar to flower breakage. The virus has been diagnosed in Iraq and there are 4 strains that depend on the basis of serological and biotic properties as well as the migration of the protein coat. Another study showed that most strains of Group 1, A1 and B1 do not cause symptoms on the tobacco plant, but they infect species of Vigna spp. Systemically and some of them give localized stains on tobacco with a yellow color, while the strains of Group 11 have local and systemic stains on tobacco and are Very severe to mild leaf distortions follow depending on strain and environmental conditions [15].

2.1.2 Cucumber mosaic virus classification

CMV (CMV) is related to the genus Cucumovirus, group Cucumoviruses, family Bromoviridae and genus Cucumovirus [16–20].

2.1.3 The size and gravity of the virus

The virus particle is spherical, ie, Isohedral, with a diameter of 28–30 nm [21]. It refers to the group of viruses of similar dimensions, Isometeric Viruses.

The genome consists of three segments of the filamentous DNA that are single-stranded and the total number of nucleotides entering its composition reaches 8621 nucleotides [20]. The largest piece is (RNA-1) which has a molecular weight of 1.3x106 Dalton and consists of 3389 nucleotides, the second piece (RNA-2) has a molecular weight of 1.1x1060 Dalton and consists of 3035 nucleotides and the third piece (RNA-3) is smaller with a molecular weight of 0.8x106 Dalton It is composed of 2197 nucleotides [22].

Sill et al. [23] found that CMV particles were spherical in shape and 35 nm in diameter. Also Scott [24]; Smith [25] and Matheuos [26] have noted that they are spherical particles with a diameter of 28–30 nm.

Indicated that isometric particles that are polyhedral spherical (**Figure** 2) and have a diameter of about 30 nm [27, 28].

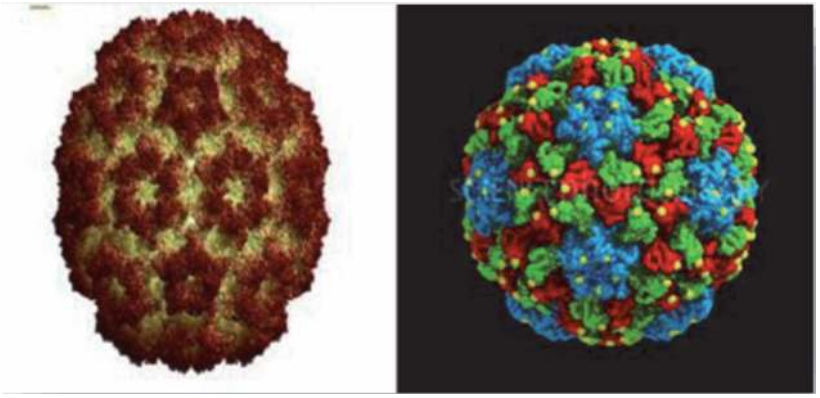


FIgure 2.
CMV shape under electron microscope.

2.1.4 Host range

It has been indicated by many researchers that the CMVhas a wide range of hosts. Also, Price [29] recorded the presence of 119 plant species of the virus, which belong to 40 families, and Nelson and Tuttle [30] mentioned that there are numbers of plants that exist. In Yama, Barisona, it belongs to many different families and is one of the important families of the virus, which are Pig weed, Wid beet, Nettlelleaf goosefoot, Lambis quarater, Saf flower, Winter synash, Periwinkle, Grownd cherry, Sowthistle.

CMV virus has the characteristic of spreading in all parts of the world because it has a wide host range and it works on infecting many types of ornamental trees and vegetables in addition to cucumbers [31].

2.1.5 Transportation methods

Insects are one of the most important means by which plant viral diseases spread in nature, and aphids have the main role among the insects that are capable of transmitting and spreading viruses, Maramorosch et al. [32] confirmed that the CMVis transmitted in an unsustainable manner by more than (60) types of aphids and that the transmission capacity varies according to the type of aphids, type of the host plant, and it is noted that the virus strain is more transmissible than other strains, and they also indicated The virus transmitted in this way will be completely lost within only two hours. El-Sayed et al. [33] found that it is easily transmitted CMV from the green peach *M. persicae* (**Figure** 3**A**) and *A. fabeae* (**FIgure** 3**B**).

This virus is transmitted by the seeds of the wild cucumbers that are adjacent to the cucumber fields, as well as the mulberry seed which is at a ratio of about (10–40%) in addition to that it is transmitted by the seeds of many jungles of annuals and is a small percentage. Transmission is accomplished by mechanical means, by means of contamination by hands, as well as easily by agricultural tools [34]. Franchi et al. [35] indicated that this virus is seed-transmitted, It can occur in 19 plant species, including some jungle plants. Mathews [26] has shown that this virus has the ability to survive in soil for a period of at least 12 years.

El-Sayed et al. [33] assert that CMV is not transmitted by the seeds of cultivated cucumbers, but that infection by viral infection leads to a reduction in seed weight by 23.5%.

2.1.6 The importance and spread of CMV

The CMV is one of the most important and widespread viruses, as it spreads in all regions of the world, and has a wide range of families, the most important of

Figure 3.
Transmitted CMV by aphis. (A) Myzus persicae, *(B)* Aphis fabeae.

which are lettuce, celery, carrots, parsley, beets, walnuts, beets, spang, and many types of bush and ornamental plants [36]. It has been indicated that the CMV infects more than 1287 plant families, which belong to 518 plant genera and 100 species, CMV virus causes many economic losses to many crops [18, 37] and my understanding 2006).

It was recorded in America by Price [38]. In neighboring countries, it is considered one of the most important viruses that infect vegetable crops as well as ornamental plants, namely Jordan, Iran, Syria and Turkey [39].

2.2 Diagnosing CMV

Diagnostic methods have been used in a variety of ways to detect CMV virus by using a group of detector plants, which in turn give clear systemic symptoms, the most important of which are tomato *Lycopersicon esculentum*, watermelon *L. cucumis* melon, *Citrulli lanthatus* L. and cucumber L. Cucumber *sativus* and L. *Cucurbita pepo*, as well as there are plants that form symptoms in the form of local spots on arugula, barley and cowpea plants. This method has been adopted to diagnose the virus by many researchers.

There are many other methods through which the diagnosis is made by using anti-serum with many serological techniques that have been used to detect infected samples, the glass slide clumping method, double diffusion of clots, and ELISA tests were used [40].

Hu et al. [41] used the RT-PCR test, the reverse transcription-polymerase chain reaction, as well as the dot-blot assay to be detected, and the Tissue-Blot Immunoassay (TBIA) technology was also used [42].

It was also detected by cytofluorimetric fluorescence [43].

Immunostrip tests were used in the diagnosis of viruses and were successful, including CMV virus [44]. Use of protein electrophoresis on an acrylamide gel to detect the virus [45].

2.2.1 Polymerase chain reaction PCR technology

RT-PCR method is considered one of the most important rapid techniques and is effective and decisive in order to diagnose and determine the type of strain and know the difference between viruses that are similar and are used in disease control as well as control breading and mitigate the epidemic with all plant viruses [46]. A fast and sensitive method, which is inferred by detecting viruses in comparison to other tests.

This technique can be used to detect very low concentrations of viruses in plant tissue, and because of their accuracy as well as their sensitivity, it has been used to detect viruses and thus to identify strains successfully.

These specifications have led to applications in medicine, forensic analysis, agriculture, and mutation analysis in eukaryotic organisms. PCR technology depends on the replication of pieces of DNA by a type of DNA polymerase that is heat resistant outside the body of the in vitro to millions of copies by the presence of the primer. It is linked according to the sequence that completes it on the DNA Template with the presence of a sufficient amount of dNTPS, and the cloning and elongation reactions remain in a sequential manner, depending on the temperature change. As for the RNA, the previous steps of replication are tracked, which is converting it into cDNA by the method called RT-PCR. Same steps for replication and elongation of DNA.

2.3 The role of resistance with bio-agents and plant extracts in limiting the harm of viral diseases

2.3.1 Stimulating the systemic resistance of plants against plant pathogens by bio-agents

The plant can resist a "pathogen" or several pathogens through structural or biochemical defenses that help the plant to inhibit the pathogen. The plant defense mechanisms are a result of the activity of the gense resistance genes responsible for plant defenses [47].

Many different biological factors have been used in the resistance of pathogens, especially viral pathogens [48]. The first knowledge of induced resistance was recorded in 1933 by Chester, and the first to use this term was Ross in 1961 who inoculated the lower leaf of tobacco plants sensitive to (TMV) Tobacco Mosaic Virus and this induced resistance in the upper leaves against TMV [49].

Induced resistance is the resistance that is based on the structural and chemical defenses that are induced after inoculation with an unsatisfactory or pathogen incompatible with the plant host, so this type of resistance develops systemically as a response to the presence and settlement of PGPR bacteria. This resistance shows specialization in stimulating resistance.

Induced Systemic Resistance (ISR), unlike Systemic Acquired Resistance (SAR), is not related to the expression genes of the pathogen-related proteins of the acquired resistance. The induced resistance is either local or systemic and the meaning of resistance. Localization is the resistance that occurs by entering the pathogen into the tissues of the plant and thus results in the production of phytotoxins, as well as the grouping of two lignins so that they lead to strengthening the walls of the cells and difficult to penetrate by the pathogen or because it leads to the death and destruction of plant cells and their dehydration, which is called the hypersensitivity reaction (HR), GM plants in which the CP protein coat accumulates from the tobacco mosaic virus TMV are resistant to infection with TMV.

Resistance by the CP gene has been demonstrated against CMVCMV, clover mosaic virus AMV, potato virus PVX, tobacco streak virus, and other viruses [50].

Many theories have been developed to explain the stimulation of plant growth and resistance by these factors, and the most common is the secretion of antibiotics and the production of compounds that compete with chemical elements needed by the pathogen in its development, as well as stimulating resistance genes with these materials and editing the work of the operator genes by disengaging them from the repressor protein molecule. It is reflected in the manufacture of anti-virus materials that may be proteins, including enzymes linked to the virus and that prevent the release of DNA [51, 52].

It was found that *Pseudomonas fluorescens* and *Rhizobacteria leguminosarum* induced systemic resistance in plants against BYMV [53] tested the possibility of stimulating pepper plant resistance against PMMOV.

Defensive gene products that include peroxidase enzyme (po) and polyphenol oxidase (ppo), which are concerned with the combination of lignin and phenaline ammonialase (pal) as they have an important role in the manufacture of phenols, phytotoxins and other defense enzymes that include pathogen-related proteins (pr), for example b1,3_glucanases (pr2 family), chitinase and the lipase enzyme (pr3 family), which causes the decomposition of the fungal cell walls and thus the complete analysis of the cell when a fungal infection occurs that works to dissolve the lipoprotein envelope of the virus if the infection is an enveloped virus [54].

Several researchers have discussed the possibility of inducing cucumber and tomato plants to be resistant to CMV(CMV) as well as tomato mottle virus (TOMOV) using isolates from *P. fluorescence, P. putida* and *Bacillus pumils* [52]. The application of *P. fluorescence* induced SR in cucumber against CMV.

2.3.2 Pseudomonas fluorescens

P. fluorescens bacteria represent a large and important group of Gram-negative bacteria that are present in large numbers as well as are free to live in soil, fresh water and salty environments, especially in the vicinity of the roots due to their ability to grow on organic materials that are abundant around the roots and many other natural environments,

It has received great attention within the term Induced Systemic Resistance (ISR) due to its effect on protecting plants treated with it as it is considered one of the most important types of root bacteria that stimulate plant growth [55], and in addition it has beneficial effects to promote plant growth. And that by providing the treated plants with many growth regulators, including gibberellins, auxins, and others [56].

Al-Fahad [57] has indicated that *P. fluorescens*, when used as a bacterial vaccine, has the effect of improving or increasing and stimulating growth, which is represented by increasing the leaf area, chlorophyll, in addition to stimulating the resistance of plants to infection with the virus or reducing its multiplication within plant tissues, and this positive increase was significantly and clearly reflected on the outcome.

Al-Fahad [52] mentioned that there are several theories that explain this stimulation, including the secretion of antibiotics by the stimulus factor outside or inside the plant. P.F bacteria were used in plant resistance as inducers against CMV, and it led to a decrease in the severity and rate of infection with the virus.

It grows at an optimum temperature of 20–30° C and most strains grow at 4° C [58]. Kazenpour [59] found that treatment in P.F with rice seed reduced the severity of rice sheath blight by 10.5%, while in comparison it was 52%.

2.3.3 Spirulina platensis

We have been heading to thinking about resistance to viruses by stimulating resistance against it, due to the difficulty of controlling it by traditional methods. One way to enhance plant resistance against viruses is the use of the algae *S. platensis*.

It was recently classified by Ali and Saleh [60] and Kewi et al. [61] as a type of bacteria, **Figure 4**.

Kingdam (Domain): Eubacteria, Bacteri

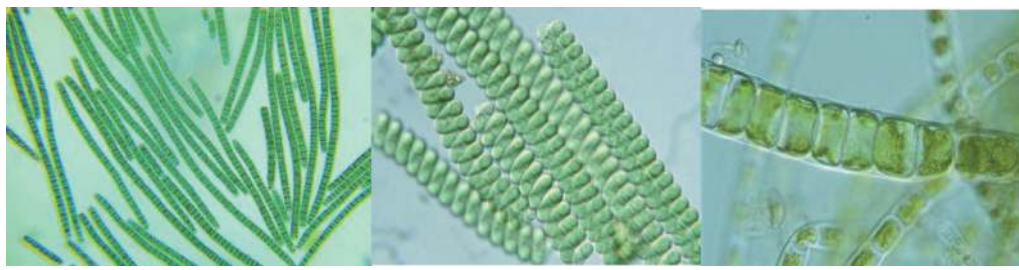


Figure 4.
S. platensis *form under a microscope.*

Phylum: Cyanobacteria
Order: Spirulinalies
Family: Spirulinaceae
Genus: *Spirulina*
Species: *Platensis*

Algae are ancient discovered organisms that date back to fossils about 305 billion years ago [62]. Its importance for health and the prevention of various diseases has been mentioned [63].

Spirulina is a bluish green algae that has a filamentous and spiral shape and its taxonomic position is unique because it combines Autorrphic [64], which is common in eukaryotic cells as well as bacteria and has high nutritional value, for example, vitamins B1, B2, and A [65]. The chemical composition is from proteins 55–70%, carbohydrates 15–25%, essential fatty acids 18%, minerals, vitamins, dyes, for example carotenoids and chlorophyll [60]. It was recently classified as one of the rare edible bacteria due to its low concentration of purine [66]. *S. platensis* is widely used as a nutritional supplement for human health, as well as animal feed, and its importance lies due to its high protein content of 60–70% and its high concentration of essential amino acids, fatty acids, minerals, vitamins and antioxidants [66, 67].

Blinkova et al. [68] showed that Sulfolipid extracted from *S. platensis* inhibits the activity of HIV and also consists of many amino acids and sugars and is considered essential compounds in addition to the micro and macro elements that have the essential role to improve plant growth [69].

Blue-green algae are highly efficient in producing a wide range of antibiotics that have a direct effect in inhibiting the growth of pathogenic bacteria. The anti-bacterial effect of marine algae is not limited to bacteria but also has an anti-viral effect [70]. Green algae have a high efficiency to produce a group of antibiotics and have a direct effect in inhibiting the growth of pathogenic bacteria P.sp., which is resistant to antibiotics. It is also considered one of the most important organisms due to its wide range and bioactivity including: This effect on viruses is inhibitory [71].

The results obtained by Buter and Hunter [72] have supported the addition of extracts consisting of seaweed to improve the growth and productivity of the plant, and that the process of foliar spraying through these extracts has led to an increase in vegetative and productive growth and the reason is to provide essential nutrients that the root cannot provide. Mishima [73] showed that *S. platensis* has the ability to inhibit the multiplication of many viruses.

Spenille and others [74] indicated that algae have significant effects as they increase the resistance of plants against disease, when added to the plant, and it is one of the most important organic sources used in plant production. The anti-effect of marine algae does not depend on the bacterium only, as it has an anti-viral effect [70]. Algae are important biological groups due to their wide range and biological effect, including the inhibitory effect against viruses [71]. The treatment of pepper plants with *S. platensis* had a positive effect in reducing the severity of ToMV mosaic virus, by reducing the phenotypic symptoms, the activity of the peroxidase enzyme, and the increase in leaf area, plant height, chlorophyll percentage, and dry weight of the shoots [75]. The United Nations Food and Agriculture Organization (FAO) as well as the independent governmental organization, www.iimsam.erg [76], recommended and emphasized the necessity of using SP as a basic and main tool to combat malnutrition in the world as well as to help achieve sustainable development. In India, some villages were working on the use of blue-green algae to improve the quality of saline soil in Samphar in Rajasthan, it is dried and used as fertilizer for crops such as wheat and barley. Gupta et al. [77] used some types of blue-green algae (*Arthrospira subsalsa & Spirulina Platensis*) with bio-fertilization and mitigation of salinity damage.

2.3.4 Medicinal plant extracts

Alternatives to chemical pesticides such as plant extracts have been used to protect plant production from diseases caused by fungal, bacterial, viral and nematode organisms, as they cause great losses to the economy during the growing season or in the post-harvest stages.

It was found that the extracts of pepper, geranium and datura plants have the ability to induce resistance to viral infection in the paper that was treated before inoculation with the virus, as well as act as infection inhibitors when mixed with the virus, and it was found that they contain compounds of partial weights, low and high that have effectiveness against viral infection [78].

2.3.4.1 Sesabania spp.

The *Sesabania spp*. Belongs to the family of the leguminous Fabaceae or Leguminosae, and the leaf is a main compound, the stamens have many flowers and the fruit is a pod and contain a group of seeds. Therefore, many countries paid attention to this plant. Ndungiu [79] mentioned that there are many international institutions in East African countries that they have conducted a complete and comprehensive survey of the varieties that spread in countries, Malawi, Zambia, Botswana, Namibia, Zimbabwe.

Sesbania sesban Linn otherwise known as "Sesban" (**Figure 5**) is one of the six species of the genus Sesbania that is commonly found for cultivation in the tropical region of India. The plant is widely cultivated for its ability to stabilize nitrogen and wind shade, obtained good medicinal importance according to ethnomedical claims [80].

The plant contains many phytochemicals, including saponins terpenoids, which are fat compounds that are composed of the bindings of B-amyrin, Licoric acid and Glabrolide, Among them is Isoprene, Osiprene called Penta-hydrocarbon, Glycyrrhetol, Glycyrrhizin and Liguiritic acid. The effective natural products present in the plant S. sesban as a means of finding alternatives to the chemical pesticides manufactured because of the effective compounds this plant contains, which include phenolic compounds, including saponins [81].The leaves of the plant contain many important components that are found within the chemical components of the plant, including Alkaloidl, Flavonid, Tannius, Terpenad.

The use of this plant to get rid of pathogens by using it in the form of an alcoholic and aqueous extract or in the form of powder, and it also has high efficacy

Figure 5.
Sesbania sesban *plants.*

against fungi and bacteria and that these are isolated from Sespan, which have the effect of flavonoids [82, 83].

2.3.4.2 Albizia spp.

Albizia is widely spread in the world and the scientific name of Albizia is (L) Albizzia lebbeck and belongs to the family of Leguminosae. Its leaves are large and a fragrant group of yellow flowers and long seeds. It belongs to the family Leguminosae and is found in tropical Asia and is widely cultivated in other tropical and subtropical regions including Malawi [84]. Its flowers, bark, roots and stems are all used in medicine [85].

Albizia is an important plant as an important source of pesticides and because it contains important chemical compounds such as Saponins Alkaloids, Flavonids, Tannius [86]. The common name is bacha chin, ficus and acacia, and it is widely cultivated in the regions of tropical Asia, Africa and subtropical Asia [87].

It has many uses, including medicinal, environmental and economical, and is used as shade trees as well as wood production, and is considered to be of natural importance [88]. Its seeds contain lectins that are toxic to humans and animals.

2.3.4.3 Glycyrrhiza spp.

The (Licorice) *Glycyrrhiza glabral,* which belongs to the Leguminosae family, and one of the most famous genera is the Glycyrrhizin plant, which contains 20 species. Glycyrrhiza, which means sweet veins in Greek.

Licorice contains many chemical compounds, as it is characterized by the availability of chemical compounds that have a sweet taste, and it contains Glycyrrheiel, Liguoric acid and flavonoids, including Glabridin and Glabrin [77]. Glycyrrhizin with its acid is the most important ingredient, it has an activity similar to that of steroid hormones, i.e. plant hormones are formed and thus lead to an increase in protein formation and an improvement in the indicators of vegetative growth [89].

The plant spreads in Asia Minor and Turkmenistan in the east to Spain in the west, which are its original home and also because of its medicinal value It has become cultivated on large areas in France, Spain, Germany, Russia, Italy, China, Turkey and America as well as the Arab Gulf and Iraq [90].

Al-Ajili [91] emphasized that licorice has high concentrations of amino acids as well as carbohydrates and other important elements, which leads to its great importance in nutritional uses. Licorice, G. glabral, a type of the Leguminosae family, is of great medical importance, as it is used for treating stomach irritation, coughing, hoarseness, bladder and kidney inflammation, as well as gout and muscular rheumatism, the autumn season are among the best dates for collecting licorice, and the reason is because the active substances are more abundant in this season, and Autumn is the best time to collect licorice because the active ingredients are more abundant [92].

Chemical analysis of licorice extract revealed that there are phenolic compounds, terpenes, gummies, starch, and flavonids extracts due to phenolic compounds for resistance to the microbes *Candida albicans*, *Staphylococcus aureus*, and Mycobacterium semegmutis, and the effect of extracts of ten species, including licorice, which showed very high efficacy in inhibiting TYLCV, reaching 100% for all concentrations used [93].

Licorice leaves are used to extract antimicrobial agents such as Pincembrin and Licoflavanone [94]. And the extract is used to protect against atomic radiation, as there is in the extract the substance alpha-amyrin that combines with the substance

Methyluracil to form a compound that works to protect against atomic radiation, Maliuta [95]. And the World Health Organization (WHO) stated that licorice extract treats laryngitis and is used as an expectorant to treat colds and coughs, as well as bronchitis, to treat rheumatism, to protect the liver from poisoning and to arthritis, and to treat tuberculosis [96]. The active ingredients are Glycyrrhizin, Flavon Glycosides, Liquiritoside,Isoliquiritoside, Sucrose, Dextrose, Starch, Proteins, Liposomes, Resin, Asparagine, Sterilic Oil, Saponins [97].

3. Practical experiments followed in diagnosing and controlling cucumber mosaic virus

3.1 Collection of infected plant samples

As provided in [75].

3.2 Laboratory experiments

As mentioned in [52] with some modifications.

3.2.1 CMV molecular diagnostics

The local isolate mentioned in paragraph 3.1 obtained by Prof. Dr. Maadh AbdulWahab Al-Fahad was molecularly diagnosed by means of the technique of polymerase chain reaction, RT-PCR and according to the following steps, which were obtained from the producing company (BiONEER) for the kit (Diagnostic kit) with some modifications which included:

1. Isolation of RNA
2. Solutions used to isolate RNA
3. Isolation of Total Viral RNA
4. Measurement of extracted RNA purity and concentration
5. DNA replication by using RT-PCR technique

3.2.2 The solutions used in the migration process

A. SB solution with a strength of 1X.

B. B-Loading buffer 10x magnification.

Preparation of the Agarose Gel and Electrophoresis Procedure:

3.3 Prepare spirulina, bacteria and plant extracts

3.3.1 Spirulina

The nutritional supplement represented by *Spirulina platensis* was obtained from the Malaysian company DXN, as 24 spirulina tablets were taken, equivalent to 6 g of

spirulina, crushed and placed in 54 ml of distilled water, each 1 g, 9 ml of distilled water was placed [75].

3.3.2 PF bacteria

An isolate of *Pseudomonas florescence* (pf) was obtained from the biocide Bactvipe provided by International Panacea Ltd. Sterilized and ready, the hood was sterilized and the medium was incubated in the incubator for a period of (72 hours) at a temperature (38° C). It was observed that the growth rate of bacterial colonies reached 100% of the dishes used in agriculture.

The dilution was used (10–4.10-5.10-6.10-7) and the fastest growing dilution and the best was the 6–10 dilution of the biological product where the plate was taken and added to a baker containing 500 ml distilled water to soak the seeds in it for 2 hours.

3.3.3 Extracts (sespan, albizia, and licorice)

A water-based extraction method was used to obtain the active compounds in the licorice plant, and this method was done according to Al-Ajili [91].

3.3.4 CMV virus extract

The method mentioned by Qasim and Ali [98].

3.4 Determination of amino acids of plant extracts

Amino acids were estimated based on the method presented by Scriver et al. [99] and according to the conditions attached in table below:

Time	Methanol A	Buffer B	Flow rate
09	20	80	1 ml/min
10–13	40	60	1 ml/min
14–25	90	10	1 ml/min

3.5 Determination of active compounds in the dry leaves of medicinal plant extracts

The active compounds were diagnosed in the dry leaves of Sispan, Albizia and licorice roots by using the High Performance Liquid Chromatography (HPLC) device. The dry leaves of Albizia, Sisban and licorice roots were extracted according to the method [100, 101]. The concentration of the active compounds was measured using the following model concentration equation:

$$\text{Sample concentration mg/ml} = \frac{\text{Space package form}}{\text{Area of the standard solution}} \times \text{Standard solution concentration} \times \text{Number of dilutions} \quad (1)$$

3.6 Field experiment

3.6.1 Plant varieties used in the experiment

The plant varieties referred to in the **Table 1** below and the companies producing them were obtained from the local markets for the sale of agricultural supplies.

3.6.2 Methods of treatment with materials used in the experiment

A. Albizia: The seeds prepared in the experiment were treated according to the scheme, by drenching (20) seeds for each line from the extract, for a period of (2) hours, and then sown in the field directly according to the scheme prepared for the experiment.

   As for the second treatment, it was sprayed only on the third and fourth real leaf, and after grinding the leaves of Albizia (5) grams per liter of distilled water only. The third treatment was at the beginning of flowering in the same way as the second treatment.

B. Licorice: Licorice was treated in the same way as mentioned in Paragraph A, but for the roots.

C. Sespan: I prepared the treatment of the extract of sespan leaves in the same manner mentioned in Paragraph A.

D. Spirulina: The moss suspension was prepared by adding 1 g of *S. platensis* powder to 9 ml of sterile distilled water and the volume was completed to 1 liter of distilled water and soaking 20 seeds in it for 24 hours. The second treatment was spraying at the flowering stage. The third treatment was at the beginning of flowering in the same way as the second treatment.

E. P.F bacteria: The seeds that were prepared for cultivation were treated according to the scheme of the experiment, by soaking (20) seeds in the concentration (106) prepared in advance in the laboratory experiment, for a period of 24 hours. The second treatment was spraying at the flowering stage. The third treatment was at the beginning of flowering in the same way as the second treatment.

3.7 Measuring indicators of CMV infection

3.7.1 Calculate CMV infection ratio

It has been calculated per treatment according to the following equation:

$$\text{Infestation\%} = (\text{Infected plants number})/(\text{Total number of plants}) \text{ X } 100 \quad (2)$$

NO.	Name of varieties	Type of varieties	Producing company	Origin	Symbol
1	Superina	Hybrid	Nickerson-Zwaan	Holland	A
2	NAJIM	Hybrid	Seminis vegetable seeds	Chile	N
3	GHAZEER	Hybrid	Seminis vegetable seeds	USA	G

Table 1.
The plant varieties used in the experiment.

3.7.2 Measuring the severity of CMV infection

A guide (**Table 2**) to the severity of the infection was made by Prof. Maadh Abdel Wahab Al-Fahad, consisting of six degrees to know the development of the apparent symptoms on the plants. It was calculated by the number of affected plants in terms of the degree of each plant [102].

$$\text{Severity of infestation\%} = (0 \times 0 \text{ grade of plants number} + \ldots + 5 \times 5 \text{ grade of plants number})/(5 \times \text{examined for plants total number}) \times 100 \quad (3)$$

Photo	Description	The degree of injury
	Healthy	0
	Light mosaic	1
	Severe mosaics on the leaves	2
	Severe mosaic and beginning wrap the leaves	3
	Intense yellowing, mosaic and severe leaf curl	4
	Severe mosaic and leaf wrap With deformations and shortening of the leaf space	5

Table 2.
Disease index of severity of CMV infection, by Dr. Maadh Alfahad.

The experiment measurements also included the following characteristics: Measuring the amount of chlorophyll, measuring the leaves area, measuring growth and outcome indicators, estimate the amount of total yield/plant, measure the plant height (cm. plant), measurement of dry weight of shoots/g, measurement of the dry weight of the root mass.

3.8 Statistical analysis

The experiment was carried out according to the independent randomized complete block design (RCBD) and the averages were compared according to the Least Significant Defense (LSD) test at a probability level of 0.05.

4. Results and discussion

4.1 Isolation and diagnosis of cucumber CMV mosaic virus

4.1.1 Symptoms

The symptoms appeared in mosaic on cucumber plants inoculated with CMV virus after a week of inoculation and over time the symptoms turned into deformation of the leaves and their edges bending down and twisting and stunting of the plant, as well as shortening of the necks of the leaves in addition to the lack of fruit numbers, deformation and dropping of flowers, and with the progression of the infection Areas wherein turns into yellow areas and then into dead areas (Necrosis), and no symptoms of infection with the virus appeared on healthy plants protected from infection.

4.1.2 CMV molecular diagnostics

4.1.2.1 Results of RNA isolation

Isolation of DNA from the leaves of cucumber plants infected with the virus, and an appropriate percentage of RNA was obtained, as the Kit (ready-made kit) method was used to obtain RNA in appropriate quantities ranging between (12.696–7.420) nanograms per microliter and the purity was (1.71) This result came after repeated attempts because of what is known that the process of isolating RNA is more difficult than DNA because it is sensitive to cracking by the RNase enzymes (Hassan, 2004).

4.1.2.2 Results of PCR reactions

A. RNA concentration.

B. The concentration of the initiator that was used.

C. Set the appropriate program to operate the thermal polymerization device.

When using the program mentioned in paragraph (3.3.1.5) the straight segments responsible for forming the virus' protein envelope were enlarged with the fourth primer (Forward 4: H), where the reaction showed very clear Bands with size (207 bp), and as They are shown in **Figure 6**. As for the primers Forward 1,

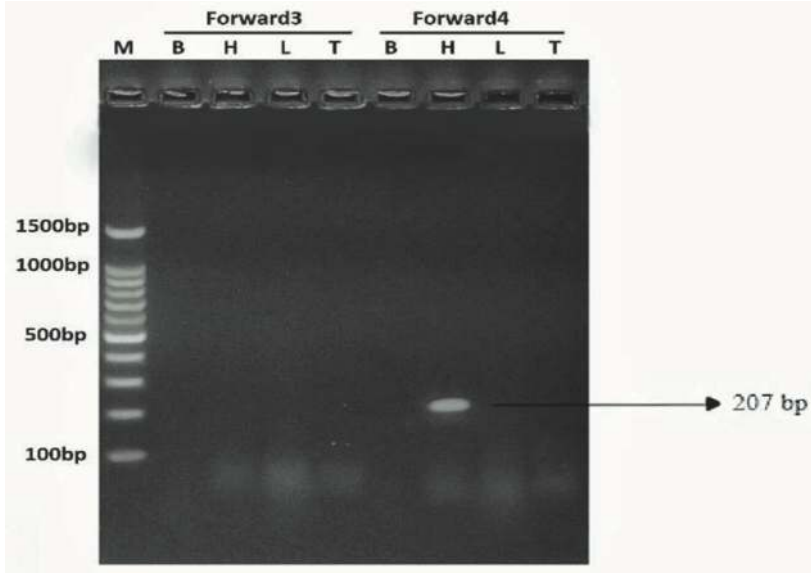


Figure 6.
A result of molecular detection of the local isolation of CMV virus for the third and fourth initiator. M: represents the volumetric index. Forward 4: H: represents the initiator of 207 bp for the fourth primer. B, L, T: Forward 3 represents the third primer. No package appeared.

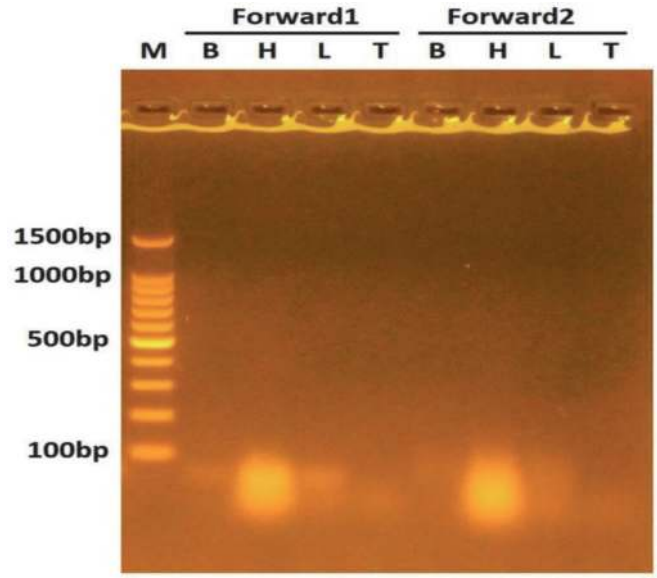


Figure 7.
The result of molecular detection of the local isolate of CMV virus for the first and second initiator. M: represents the volumetric index; Forward 1,2: represents the first and second primers where no bundles appeared; H: stands for cucumber. B: represents the pepper. L: represents tomato; T: symbolizes Al-Taroozi plant.

Forward 2 and Forward 3, they did not show any inflation as in **Figure 7**. This result of the molecular diagnostic test is similar to the findings of Shomaila Igbal et al. [103] in Rawalpindi region/ State of Pakistan, while the rest of the primers did not match, and this indicates a great convergence between the aforementioned isolation in Al-Huwaish/ Samarra district/Salahuddin with the Pakistani isolation. And a difference from the isolation in the regions (Punjab, Sind, Northwest Frontier Province, Islamabad and Baluchistan).

4.2 Evaluation of the effectiveness of some biological agents in inducing plant resistance against CMV virus in cucumber plants

4.2.1 The effect of the treatments used on the percentage of infestation to plants

The reason for the superior treatment of spirulina, P.F, and licorice (**Table 3**) may be attributed to the fact that spirulina algae produces biological compounds that have a toxic and anti-biotic effect for a group of microorganisms, including viruses.

Also, the low rate of infection of cucumber plants with CMV virus is due to the role of P.F. as a catalyst for systemic resistance against viruses by treating the seeds with the bacteria before planting. The reason for this may also be due to the fact that these bacteria induce resistance in plants against the CMV virus, and it led to a decrease in the rate of infection with the virus.

Treatments	Varieties							
	A	BEIT ALPHA	G	GHAZEER	N	NAJIM	Impact rate of treatments	
A	d-k	46.67	b-j	57.33	d-k	44.67	d-e	49.55
B	c-k	51.33	b-j	59.00	d-k	46.33	c-e	52.22
C	c-k	52.67	b-j	59.00	b-j	59.67	b-e	57.11
P.F	h-k	37.33	b-f	66.00	d-k	44.33	d-e	49.22
S	b-e	67.33	f-k	40.33	c-k	52.00	c-e	53.22
A+B	b-j	60.33	b-d	69.00	a-c	76.67	b	68.66
A+C	b-j	58.00	b-f	66.33	b-j	59.67	b-d	61.33
A+P.F	c-k	52.67	b-j	59.00	b-e	67.33	b-e	59.66
A+S	c-k	52.00	a-b	81.00	b-j	63.00	b-c	65.33
B+C	b-f	64.33	b-k	54.67	b-e	67.00	b-d	62.00
B+P.F	j-k	36.67	b-f	63.67	b-j	59.33	c-e	53.22
B+S	i-k	37.00	c-k	51.33	b-d	68.33	c-e	52.22
C+P.F	b-k	55.33	b-k	54.67	a-c	76.00	b-d	62.00
C+S	d-k	48.67	b-f	65.67	b-j	62.33	b-e	58.88
P.F+S	b-f	66.67	k	30.00	b-j	61.67	c-e	52.77
A+B+C	c-j	60.67	d-k	48.00	b-d	70.67	b-e	59.77
A+B+P.F	b-g	64.00	b-f	65.33	c-k	50.67	b-e	60.00
A+B+S	b-f	65.67	d-k	49.00	b-j	59.00	b-e	57.88
B+C+P.F	b-i	63.33	c-k	52.00	b-f	66.67	b-e	60.66
B+C+S	b-j	63.00	d-k	47.67	d-k	47.00	c-e	52.55
B+P.F+S	e-k	41.00	b-j	59.00	g-k	37.67	e	45.88
CO1	0.00	l	l	0.00	0.00	l	f	0.00
CO2	a	100.00	100.00	a	100.00	a	a	100.00
impact rate of var.	54.11	a	56.43	a	58.26	a		

**According to the Duncan polynomial test at the level of 5%.*

Table 3.
*The effect of the treatments used in the incidence of CMV infection % on three varieties of cucumber.**

The explanation of the superiority of the triple treatment between *S. platensis*, licorice and p.f bacteria may be attributed to its containment of most of the compounds needed by the plant to improve growth and also increase the efficiency of the photosynthesis process, and this is reflected in the inhibition of viral infection.

Or perhaps the reason for this is that *S. platensis* has inhibitory activity, which is clearly because it contains polysaccharides, cyclic peptides and alkaloids.

Al-Fahad [57], citing Jeanneus and Tetau [104], also mentioned that licorice extract contains Glycyrrhizic acid, which has medicinal efficacy and has affected the inhibition of the virus by forming hydrogen bonds linked to the virus protein or DNA or both, and this led to the limit of its effectiveness. Studies have indicated the possibility of stimulating plants to produce virus-inhibiting compounds as mentioned by Maurhafer et al. [105]. Or perhaps the reason for this is due to the fact that the amino acid Thereonin present in licorice extract led to an increase in the inhibition of the virus and reduced the rate of infection, as shown in **Table 3** and also the active substance that was disclosed in **Table 4** and **Figure 9**.

4.2.2 The effect of treatments on the severity of infestation to plants

The reason may be that the compound glycirizine with its acid present in licorice, which has an activity similar to that of steroid hormones, i.e. it is a form of plant hormones and leads to an increase in the formation of proteins, as shown by Tyler [89]. Also, Al-Janabi [93] concluded that licorice, which showed a very high efficacy in inhibiting TYLCV, reached 100% and protected tobacco plants against infection with TMV virus for 30 days.

This may be due to the fact that licorice extract contains terpenes, phenolics and starch, as well as resins, and flavonoids are due to phenolic compounds that resist many microbes.

The superiority of the interaction treatment between *S. platensis* and PF bacteria can be explained by the fact that the algae possesses materials rich in proteins, vitamins and minerals in addition to fatty acids, polyphenols and sugars and contains pigments such as carotenoids and chlorophyll that stimulate the growth and resistance of plants to pathogens and this is consistent with what he referred to. Abbasssy et al. [106] and Usharani et al. [107]. Also, the results of this study were consistent with the findings of Kim [55] that PF bacteria induce systemic resistance (ISR) of plants against diseases, as the study agrees with what Murphy and others [108] and Ryu and others [109] and EL-Dougdoug et al. [110] have indicated, with different mechanisms, including that the PF bacteria that are isolated from the roots are of great importance as growth stimuli and are considered as biological control agents for plant pathogens. These bacteria were used to induce resistance to plants against viruses or the secretion of multiple enzymes that help in analyzing the

No.	Plant name	Compound name	Concentration
1	Albesia	Keamferol	21.61
2	Licorice	Apigenen	76.053
3	Sespan	Gallic acid	1.196
		Qurcetine	3.402
		Ellagic acid	6.181

Table 4.
The effective compounds of medicinal plant extracts.

protein coat of the virus and also help produce Phytoluxins, which in turn are defensive compounds within plants [111].

4.2.3 The effect of used treatments on ratio of chlorophyll \ Spad, Leaf area/cm^2, plant length/cm and Yield amount/g, of three varieties of cucumber infected with CMV virus

The reason for the superiority of the treatment of spirulina, PF bacteria and licorice root extract may be attributed to the fact that the treatment with bacteria has a great role in stimulating the systemic resistance of plants and thus reduces the rates of effect of the virus on the amount of chlorophyll. The varieties, and also the tolerance to infection with the virus, may explain this effect that the bacteria PF leads to an improvement and increase the stimulation of growth, which is reflected in the increase in the amount of chlorophyll positively by increasing the chlorophyll.

Or, perhaps the reason for this is that it has the ability to form proteins that inhibit the replication of the virus by stimulating resistance genes in the plant that are associated with the virus and prevent the release of DNA, and thus the replication of the virus fails and is positively reflected in the amount of chlorophyll.

Several studies have shown that *S. platensis* contains growth regulators, the most important of which are cytokines that contribute to increasing chlorophyll synthesis, as well as compensating for the imbalance of nitrogenous bases as a result of infection with the virus. It was referred to by Mao and other [112].

The reason for the superiority of the variety *S. platensis* can be attributed to its possession of genes that carry resistance to the effects of the virus from breaking down chloroplasts, and is consistent with what was mentioned by Al-Fahad [57].

The difference in the chlorophyll percentage may be due to the genetic factor of the genes for resistance, because whenever they excel, they increase the percentage of resistance and thus reduce infection or reduce the (Virions) necessary for the formation of infection and lead to an increase in the chlorophyll percentage of the plant, and this is in line with what was mentioned by M. Others (2011).

This may be due to the fact that PGPR bacteria prevent the harmful effects of many different pathogens such as bacteria, fungi, nematodes, viruses, and produce materials that inhibit the growth of pathogens and have no harm to plants by providing the iron element that is necessary for the growth of these pathogens by Sidrophores and production Antibiotics [113].

Also, treating the seeds with soaking during planting increases the continuity of growth despite their injury, which makes the plant tolerant of the primary infection, and this is consistent with what Hassan and Jumaa [114] found that the significant increase in the amount of chlorophyll is caused by the soaking and spraying of growth stimuli.

Al-Ani [115] also indicated that licorice roots are used in the form of vegetable fertilizer because they contain nitrogen, so this may have been directly reflected in the increase in the amount of total chlorophyll, as nitrogen is of great importance in plants and this importance is through its entry into the building of many compounds necessary for growth And the continued growth of the plant, and introduces the building of photosynthesis pigments and the formation of energy compounds (NADH2, NADPH2, ATP) and bermidine and purine bases for nucleic acids and the formation of cell membranes, chloroplasts and mitochondria [116] (**Figure 9**).

Since it focuses on nucleic acids, it may contribute to compensating the plant for what was damaged by the presence of the virus and used the DNA for its benefit, and the reason may be that the amino acid Thereonin present in licorice extract led to an increase in the inhibition of the virus, unlike other compounds that were discovered and were in lower proportions From thereonin, this was reflected in the

increase in chlorophyll because the plant assimilates it and uses it in protein syntheses and helps to increase the concentration of chlorophyll and achieve the highest level of photosynthesis and thus increase the vegetative growth and the amino acids are chelating substances that increase or improve the transport of nutrients [117] as shown in **Figure 9** as well as the active substance Apigenen, which was detected with licorice extract, has the greatest effect in increasing chlorophyll.

Perhaps the reason for the superior treatment of algae, bacteria and licorice is due to the fact that *S. platensis* contains phosphorous, which contributes to an increase in the vegetative system as well as the leaf area and is reflected in the plant's ability to absorb nutrients and this is positively due to the increase in the products of photosynthesis for the manufacture of foodstuffs.

The licorice extract is rapidly penetrating inside the leaf tissue cells, thus causing an effect on plant sensitivity or by influencing the virus replication process.

The reason for the plant height may be attributed to the algae *S. platensis* containing the nutrients that are sufficient for the seedlings to grow in the first stage in a strong way and thus bear the infection, because it contains many growth-promoting substances such as amino acids, organic acids, oxins, vitamins and cytokines, which have the most important role in the growth process and activating enzymes. It stimulates plant growth and also stimulates cell division and achieves the process of escaping from disease, and this is consistent with what was mentioned by Abdel-Hafez [118] and Kazem and Hadi [119].

As noted by Verdial and others [120], In the event that the freesia plant was sprayed with licorice extract, this increased the height of the plant, in addition to the number of flowers.

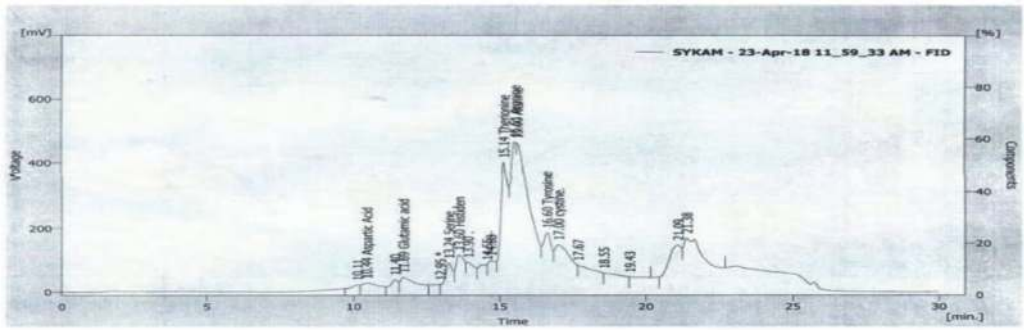
According to the results of the analysis mentioned in **Figure 9** that the components of licorice root extracts contain amino acids and high concentrations, such as Thereonin, which leads to its great importance in resistance to cultivated plants, as well as the active substance Apigenen, which was detected with licorice extract, which may have the greatest effect in increasing Dry weight. Amino acids are used in the process of regulation and balance in the root zone when the plant is exposed to a deficit in water or high salts in the root zone and glycine acid is the one that works to maintain the osmotic balance between the cytoplasm and the gap Subbarao et al. [121].

CMV virus affects the amount of yield by reducing the percentage of flowering of plants, causing distortions to flowers and leaves and reducing the proportion of chlorophyll, which results in reducing the size of fruits.

The explanation for the superiority of *S. platensis*, *P. fluorescens* and licorice extract is due to the possibility of a joint effect by stimulating plant growth and resisting the negative effect of virus infection, and may be due to the compounds that each contains separately and their effects, and that the algae work to increase vegetative growth as a result of their containment. On growth regulators and stimulating resistance, which leads to a higher percentage of nutrient absorption and an increase in leaf area, which in turn is reflected in the increase in plant weight, and this is consistent with what Crouch et al. [122].

4.2.4 Effect of compounds extracted from medicinal plants on virus inhibition and plant growth parameters

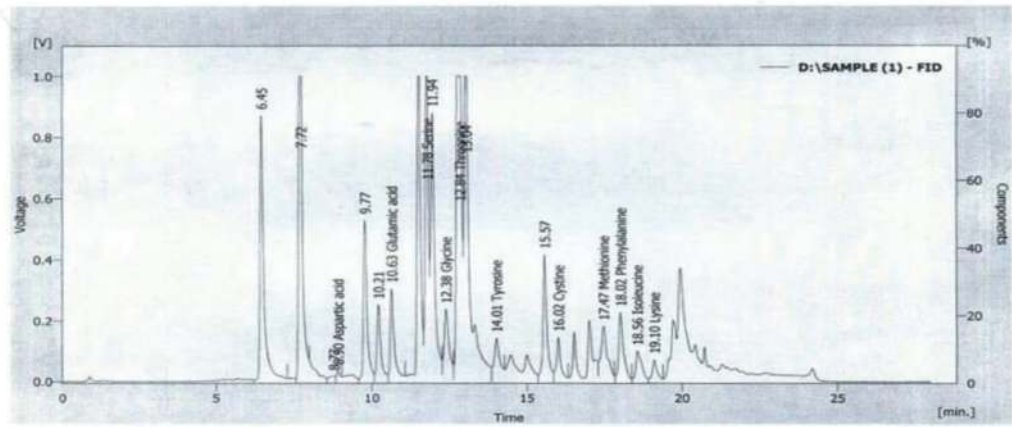
The results (**Figures 8–10**) obtained from the analysis of amino acids and the active compounds of Albizia leaf extracts, licorice and sespan showed the presence of acids: Aspartic acid, Glutamic acid Serine, Histiden, Therionine, Argnine, Tyrosine, Cystine in Albizia plant, as well as: Aspartic acid, Glutamic acid Serine, Therionine,



Result Table (Uncal - SYKAM - 23-Apr-18 11_59_33 AM - FID)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	10.112	175.293	10.766	0.3	0.5	0.26	
2	10.440	492.039	15.671	1.0	0.7	0.58	Aspartic Acid
3	11.404	332.475	22.648	0.6	1.0	0.26	
4	11.688	808.825	29.886	1.6	1.3	0.46	Glutamic acid
5	12.948	48.142	2.852	0.1	0.1	0.10	*
6	13.244	1092.065	69.410	2.1	3.1	0.30	Serine .
7	13.596	1875.715	93.010	3.7	4.2	0.38	Histiden
8	13.904	1381.326	69.789	2.7	3.1	0.38	.
9	14.552	1198.291	59.795	2.3	2.7	0.35	..
10	14.684	1337.242	72.899	2.6	3.3	0.32	
11	15.140	6473.151	373.369	12.6	16.8	0.30	Therionine
12	15.512	6259.813	435.984	12.2	19.6	0.27	Argnine
13	15.600	12976.229	431.934	25.3	19.4	0.48	Alanine
14	16.604	3250.720	152.782	6.3	6.9	0.43	Tyrosine
15	17.004	4286.765	114.048	8.4	5.1	0.72	cystine.
16	17.672	1757.426	47.065	3.4	2.1	0.84	
17	18.552	666.430	19.658	1.3	0.9	0.72	
18	19.428	118.294	5.018	0.2	0.2	0.68	
19	21.088	2540.976	91.996	5.0	4.1	0.46	
20	21.384	4252.739	109.760	8.3	4.9	0.65	
	Total	51323.957	2228.341	100.0	100.0		

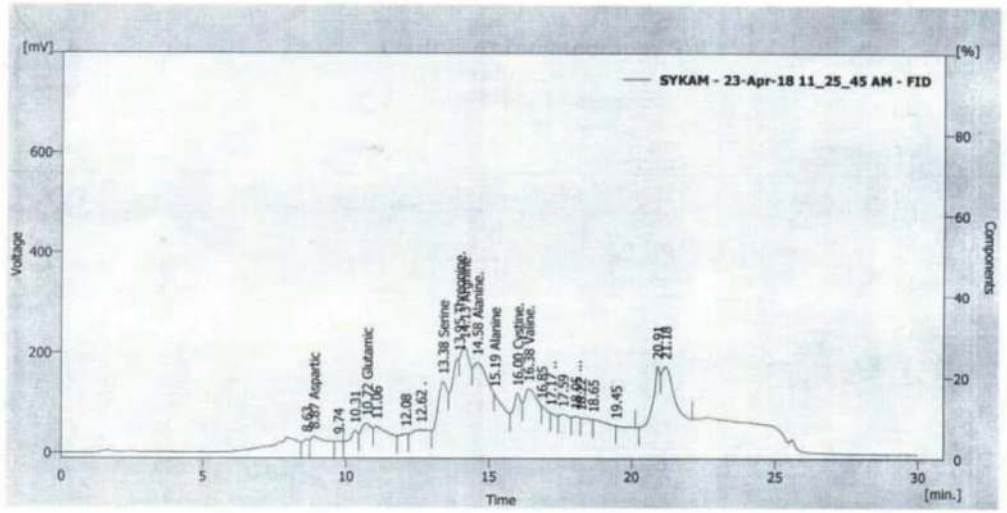
Figure 8.
Detection of the presence of amino acids and their values in Albizia leaves extract A. lebbeck.



Result Table (ESTD - D:\SAMPLE (1) - FID)

	Reten. Time [min]	Response	Amount [uL]	Amount [%]	Peak Type	Compound Name
1	6.452	7350.263	0.000	0.0		
2	7.716	8954.984	0.000	0.0		
3	8.772	47.517	0.000	0.0		
4	8.900	113.937	4.701	0.9	Ordnr	Aspartic acid
5	9.772	4093.942	0.000	0.0		
6	10.208	1959.351	0.000	0.0		
7	10.632	2271.122	67.674	13.2	Ordnr	Glutamic acid
8	11.776	6112.431	62.529	12.2	Ordnr	Serine
9	11.936	5766.347	0.000	0.0		
10	12.384	1442.747	9.660	1.9	Ordnr	Glycine
11	12.836	11593.081	125.945	24.6	Ordnr	Threonine
12	13.040	11549.287	0.000	0.0		
13	14.012	861.197	10.504	2.0	Ordnr	Tyrosine
14	15.572	3248.922	0.000	0.0		
15	16.020	1024.908	17.012	3.3	Ordnr	Cystine
16	17.472	1406.384	75.005	14.6	Ordnr	Methionine
17	18.016	2042.658	66.342	12.9	Ordnr	Phenylalanine
18	18.560	1086.537	50.384	9.8	Ordnr	Isoleucine
19	19.104	587.899	22.743	4.4	Ordnr	Lysine
	Total		512.499	100.0		

Figure 9.
Detecting the presence of amino acids, and values at the root of licorice extract G. glabral.



Result Table (Uncal - SYKAM - 23-Apr-18 11_25_45 AM - FID)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8.632	69.325	6.439	0.2	0.5	0.19	
2	8.872	248.771	11.887	0.7	0.9	0.32	Aspartic
3	9.736	7.211	0.607	0.0	0.0	0.22	
4	10.312	294.610	20.436	0.9	1.5	0.25	
5	10.724	821.633	33.588	2.4	2.4	0.45	Glutamic
6	11.060	815.095	26.999	2.4	2.0	0.54	
7	12.080	179.680	8.849	0.5	0.6	0.39	
8	12.616	653.887	15.521	1.9	1.1	0.74	.
9	13.384	2512.265	110.045	7.4	7.9	0.39	Serine
10	13.948	2779.395	154.526	8.2	11.2	0.38	Threonine.
11	14.132	4437.083	178.273	13.1	12.9	0.46	Argnine
12	14.584	5611.450	144.335	16.6	10.4	0.77	Alanine.
13	15.192	1896.059	79.110	5.6	5.7	0.55	Alanine
14	16.004	1601.167	79.686	4.7	5.8	0.39	Cystine.
15	16.384	2878.184	87.615	8.5	6.3	0.66	Valine.
16	16.848	861.307	51.197	2.5	3.7	0.32	
17	17.172	552.412	36.674	1.6	2.6	0.28	..
18	17.592	822.672	33.127	2.4	2.4	0.45	
19	18.052	515.141	27.698	1.5	2.0	0.32	...
20	18.224	567.760	23.863	1.7	1.7	0.43	
21	18.652	602.355	19.204	1.8	1.4	0.64	
22	19.452	86.089	5.136	0.3	0.4	0.46	
23	20.908	1742.040	116.360	5.1	8.4	0.22	
24	21.176	3305.922	113.340	9.8	8.2	0.46	
	Total	33861.514	1384.515	100.0	100.0		

Figure 10.
Detection of the presence of amino acids and their values in the extract of sespan leaves P. aculeate (L).

Tyrosine, Cystine, Methionine, Phenylalanine, Isoleucine, Lysine in the Licorice plant. In addition to: Aspartic, Glutamic, Serine, Therionine, Argnine, Alanine, Valine, and Cystine in the sespan. It has shown that the active compounds in Albizia leaf extract are Keamferol and Apigenen in licorice and Gallic acid, Qurcetine, Ellagic acid in Sespan.

The highest and best percentage of licorice, represented by the amino acid Therionine, was 24.6%, as it differed from the rest of the amino acids, as the amino acid Argnine for Spanish was 12.9%, the amino acid Argnine for Albesia 19.6%, and the active substance for licorice represented by Apigenen, which gave the highest concentration of 76.053 compared to Along with the rest of the extracts, the reason for this may be attributed to their effect on inhibition of the virus and increasing the growth parameters of leaf area, chlorophyll, plant height, dry vegetative and root system, as well as reducing the severity of infection in plants because amino acids are characterized by several physiological roles in plants and are of great importance to stimulate growth and maintain The pH of cells, and since amino acids contain

two acid and basic groups as well, they lead to the expulsion of ammonia from cells and are also a store of carbon and energy.

Also, amino acids are distinguished by their ability to balance nutritional functions due to their effect on plant growth and yield due to the improvement of the original infrastructure in the cell, especially the plastids in the tissues, which improves the photocatalytic efficiency and leads to the production of more new cells that are reflected in the increase in plant height, The leaf area, as well as the yield and its components, as well as the amino acids have a role in stimulating the activity of a group of proteins responsible for enzymes for protein synthesis [123]. Free amino acids are an essential nitrogen source that is involved in building proteins and enzymes and providing energy that stimulates root and vegetable growth as well [124].

The abundance of amino acids has a great role in decreasing the osmotic voltage through which the water stress of the cell is reduced, and thus helps the cells in the process of drawing water and a set of nutrients from the medium and helps to increase the vegetative growth of plants [125]. 2002 that amino acids play a role as osmotic regulators as well as they regulate the process of ion transport, open stomata and expel toxins, a group of heavy metals and have a major role in the activity and building of enzymes.

5. Conclusions

1. The isolation of the virus that caused severe mosaic symptoms on cucumber plants with its different varieties in Al-Huwaish region is related to CMV virus, which genetically approximates the Pakistani isolate that was used in the molecular test for the virus.

2. The treatment of G. glabral root extract and *S. platensis* algae powder proved that it had a pathogenic effect on the CMV virus.

3. The ability of P. florescence to induce systemic resistance of different varieties of CMV-infected cucumber.

4. The triple complementary treatment (G glabral extract, *P. fluorescens* and *S. platensis*) was superior to the rest of the treatments in reducing the damage caused by CMV infection and raising some growth and yield parameters.

5. It has been proven by analyzing the amino acids of G.glabral licorice root extract that it contains the amino acid Therionine and the active substance Apigenen, which has the effect of inhibiting the CMV virus.

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