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MOLECULAR IDENTIFICATION AND BIOLOGICAL RESISTANCE OF THE *FIG MOSAIC VIRUS (FMV)* ON FIG TREES IN SALADIN GOVERNORATE NURSERIES

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Abstract

The study aimed at diagnostic *FMV* virus from infected Ficus carica from some Saladin nurseries based on the technique of Reverse Transcription RT-PCR. A band 300 bp was given. The result showed *FMV* similar to that found in Costa Rica, Iran, Lurstan, Japan, and also gave a band of 367pb of *FMV* RNA1 and the absence of other species. FLMAV-1 isolation was detected in fig leaf with a 352pb band and is similar in some countries. The study also included the use of *Bacillus subtilis*, *Spirulina platensis*, *Ganoderma lucidum*, (*G. lucidum +S. platensis), (S. platensis+B. subtilis*), (*G. lucidum+B. subtilis*). The treatment of *B. subtilis* + *S. platensis* for yellow and black to study the percentage of the severity of the effect of the injury which is the lowest and reached 23.0% compared with a control treatment, which gave 99.6%. The results of chlorophyll the treatment of mushrooms with moss (G + Sp) with a value of Spad 43.15 which is the highest compared to the treatment of control amounted to 30.77 Spad.

Key words : *FMV*, *FMV* RNA1, RT-PCR, *Ficus carica.*

Introduction

Ficus carica is one of the most ancient fruit trees grown in the Middle East. It is needed for its nutritional and medical value (Flaishman *et al.,* 2008). It has a high content of natural compounds including beta-carotene-producing vitamin A which has anti-oxidant and anti-Cancer and the beta-cytosterol compound, known for its ability to lower blood cholesterol, as well as the presence of a number of effective plant compounds, such as Glycosides, Arabinose and Xanthotoxol (Abdel Nasser and Mohammed, 2014). As for the area planted with figs in the Arab world, it is estimated at 155000 thousand hectares and the number of trees is 428037 thousand trees and the production is 72514 thousand tons. In Iraq, the number of trees planted 416 thousand trees and the production rate of 10.00 thousand tons, which was the latest statistic for 2013 (Arabian organization for nutritional growth 2014). The FM Virus disease is one of the most common viral diseases in fig trees in the cultivated areas of the world, causing large losses and different on the leaves and fruits and reduce the production in quantity and quality (Anofka *et al.,* 2000).

This virus is due to the taxonomic order of the genus *Emara virus*, the family of Fimovirideae and the rank of Bunyavirale (Ishikawa *et al.,* 2013) has four or six parts of the genomic RNA (Walia, *et al.,* 2014). These viruses are common in the way they are transmitted by different types of ways by known mechanism as well as about 700 types Of the Moraceae family, including figs transmitted by this mechanism (Failshman *et al.,* 2008: Datwyler and Weilblen, 2004). The virus was found in most fig trees in Iraq (Shawkat, 1982). The virus spreads widely in the Middle East and has been recorded in several countries, including Tunisia and Egypt (Nahdi *et al.,* 2006), as well as in Japan, Costa Rica, Croatia, Turkey, China, Iran and other countries.

New methods of resistance to viruses were used with natural dietary supplements and were effective because the plants had defensive means that could be biologically stimulated using supplementation as well as with the protective side, decrease in infection criteria and increase in growth indicators (Youssef, 2018). (Saliva *et al.,* 2002, Diwan: 2003). The serological tests were then

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adopted with a high degree of accuracy in diagnosis, as well as the use of microscopes as a common method to know the shape and size of the virus (Nadeem, 2005), and recently used polymerase chain reaction (PCR) technology, which is a fast, reliable and very accurate way of detecting plant viruses compared to other tests, (Makkouk and Kumari 2006). In the absence of an integrated study to diagnose and resist this virus in the region and its prevalence in large proportions and the detection of local offspring and the absence of a chemical or natural to combat it, we saw the following:

Partial diagnosis of the mosaic virus and bio-virus resistance using the dietary supplement of *Ganoderma lucidum* and the biochemical supplement of *Spirulina platensis* and *Bacillus subtilis*

Identify the type of RNA fragment that expressed it.

Materials and Methods

Samples from different nurseries of Saladin Governorate were collected from several different regions (Balad, Zuluiya, Ishaqi, and Samarra) and from different varieties of black and yellow trees, with symptoms suggesting that they were infected with the virus.

Partial diagnosis of viral *FMV* virus

1. RNA is isolation: from the leaves of figs according to the method prepared by Promega USA

2. Measuring concentration and purity of RNA extract:

The concentration and the purity of the extracted RNA was measured using a RNA concentration and purity device (Nano Drop 2000).

3. Multiplexingof *FMV* Related RNAS with RT - PCR Reverse Transcrition

The one step RT-Master mix materials supplied by Promega USA were added to the recommended concentrations by the manufacturer and the method of work as follows The RT-PCR reactions with 20 µl were performed Material Sample RNA 5 µl, Primer Forward 1 µl, Primer Reverse 1 µl, Master mix 10 µl, Mgcl 20.5 µl, Nuclease Free Water 2 µl. Put the mix in the thermocycler (Veriti type) for the incubation procedure with the primers used in the diagnosis of the virus and according to the following (Table 1).

The interaction sample was entered into a thermocycler device to incubate the presence of the primer used in *FMV* diagnosis and according to thermal profile used RT-Enzyme activation was of 37°C during 30 minutes (inverse transcription), Initial denaturation 95°C for 10 minutes one cycle, (Denaturation 95°C for 30 sec, Primer annealing 55°C for 30 sec, Extension 72°C for 30 sec) × 40 cycles; and a final 7 minute extension at 72°C one cycle. The RT-PCR products were analyzed using a 1% agarose electrophoresis gel dyed with gel Red at 1X.

The interaction sample was entered into a PCR device to incubate the presence of the primer used in *FLMAV-1* diagnosis and according to thermal profile used RT-Enzyme activation was of 37°C during 30 minutes (inverse transcription), Initial denaturation 95°C for 10 minutes one cycle, (Denaturation 95°C for 30 sec, Primer annealing 59.5°C for 30 sec, Extension 72°C for 30 sec) × 40 cycles; and a final 7 minute extension at 72°C one cycle. The RT-PCR products were analyzed using a 1% agarose electrophoresis gel dyed with gel Red at 1X.

The interaction sample was entered into a PCR device to incubate the presence of the parameters used in RNA1 diagnosis and according to thermal profile used RT-Enzyme Activation was of 37°C during 30 minutes (inverse transcription), (Denaturation 94°C for 30 sec, Primer annealing 51°C for 30 sec,Extension 72°C for 30 sec) × 35 cycles; and a final 7 minute extension at 72 °C one cycle. The RT-PCR products were analyzed using a 1% agarose electrophoresis gel dyed with gel Red at 1X.

A Treatment used in resistance *Fig Mosaic Virus (FMV)*

Brought experimental Biomaterials from Parmaceutical Sdn Bhd - DXN Malaysia is (is specialized in the production of organic supplements), which included the following materials and use bacteria *Bacillus subtilis* in treatment**:**

- Treatment of the fungus *G. Lusidum* (Ga.) + *FMV* inoculation.
- Treatment of *Spirolina platensis* mulch (Sp.) + *FMV* inoculation.
- Treatment of bacteria *Bacillus subtilis* and symbolized by research B of infected seedlings
- Treatment with fungus Ga. + Treatment with moss Sp. For infected seedlings
- Treatment with feather fungi Ga. + Treatment of B bacteria for infected seedlings
- Treatment with moss Sp. + Treatment of B bacteria for infected seedlings
- Treatment with Gaucher fungus for healthy seedlings
- Treatment of Spaghetti Sp for healthy seedlings
- Treatment of bacteria B of healthy seedlings
- Treatment with feather fungus Ga + Sp
- Treated with fungus Ga + bacteria B of seedlings

Source	Name primer	The sequence	Annealing Temp.	Target bp
Elbeaino *et al*., 2009	RNA-1*FMV*	E5-s CGGTAGCAAATGGAATGAAA-3E5-a AACACTGTTTTTGCGATTGG-3	55º	300bp
Elbeaino *et al*., 2006	FLMAV1	V1-s CGTGGCTGATGCAAAGTTTAV2-a GTTAACGCATGC TTCCATGA	59.5º	325bp
Elbeaino *et al*., 2007	FLMAV2	V2sGAACAGTGCCTATCAGTTTGATTTV2-a TCCCACCTCCTGCGAAGCTAGAA	58º	360bp
Elbeaino *et al*., 2010	FMMav	V-s AAGGGGAATCTACAAGGGTCGV-a TATTACGCGTTGAG GATTGC	58º	311bp
Elbeaino *et al*., 2009b	RNA1(RdRp)	GTTATGGCTATATATTGTGATTATTCTCAAACTTGTATGGT GTGTAATA	51º	367bp
Elbeaino *et al*., 2009b	RNA2(Gp)	AGATGTGGGAAAATCATATGCTAGACCAACTTGCAGGCTTTT	51º	527bp
Elbeaino *et al*., 2009b	RNA3(Np)	GTCATGTTGATATGTGCTGCCACACTTACACATCTTACATCATCT	51º	873bp
Elbeaino *et al*., 2009b	RNA4(Mp)	CATCTTGTTGGAAACACAATAGCTTTGGCAGATTCTATT	51º	583bp

sound

- Treatment of Sp + B bacteria for healthy seedlings
- The fig control treatment for which no treatment has been added
- Comparative control infected and did not add any treatment.

Treatment of *Ganoderma lucidum*

The seedlings are treated according to the experimental plans by adding 9 ml of distilled water with 1 g of mushroom powder and adding 1 liter of water for each seedling. The first treatment was done on 13/12/2017 and the second treatment was on 7/1/2018 and the third treatment Dated 21/2/2018.

Treatment with *Spirulina platensis* moss

The seedlings were treated as planned in the experiment by adding 9 ml of distilled water with 1 g of powdered moss and adding 1 liter of water for each plant.

Treatment with *B. subtilis* bacteria

The plants were treated according to the experiment plan by adding 9 ml of distilled water with 1 ml of bacteria and then adding 1 liter of water to each plant

Measure some effect indicators in *FMV* virus and stimulate fig plant resistance:

Calculation of *FMV* severity

The scale of the disease was calculated by calculating the number of infected plants according to the degree of each of them according to the level of disease prepared in this study. The severity of injury was calculated for each treatment according to what Mickinney (1923) reported:

Total amount of chlorophyll

The relative content of chlorophyll in the leaves was measured by a chlorophyll II meter of SPAD-502 type. Three leaves of each plant were measured from the top of the plant and each plant was treated randomly and its chlorophyll content was calculated.

Results and Discussion

Isolation and diagnosis of *FMV* virus on Fig trees

Diagnosis of *FMV* on fig using PCR technique

Results of RNA isolation

The result is that primer of *FMV* has a packet of 300bp. This corresponds to Elbeaino *et al*., (2009). The RNA4 initiator has a light package of about 600bp and the initiator target is 583bp on the Agarose gel..

The result of the FLMAV1 primer was that the virus was infected with a packet of about 352bp. This corresponds to(Elbeaino *et al.,* 2006) as shown in Figure 8, primer FLMAV2 and the FMMAV primer. The dependence on diagnosis for more than one step is a major step that reveals the accuracy of the diagnosis of viruses to adopt more than one lead in this study.

This is the first study in Iraq in terms of the molecular diagnosis of viruses, which increases its importance and that molecular studies related to the RNA is more complex DNA studies.

PCR Results

This required the use of RNA extracted from infected the plant tissue with the *FMV* virus to make multiple attempts to reach the optimal conditions needed because high of sensitivity to the reaction conditions and that the best results were obtained through the optimal level of factors which were:

Concentration of the RNA: The concentration showed 641,500 ng / µl clear band and was the best.

??**Primer concentration**: use the p mole 20

Appearance of injury	The Description	Degree
	Healthy plant	0
	light yellowing on the leaves	1
	Light yellowing with light mosaics	2
	Average yellowing with average mosaics	3
	Yellowing hard with mosaics	4
	Very yellowing with severe mosaics, deformation and reducing the area of leaf blades	5

Fig. 1: Pathological evidence of *Fig Mosaic Virus.*

$$\text{severity of infection \%} = \frac{(\text{Infected plants number} * 1) + (\text{Infected plants number} * 2) + (\text{Infected plants number} * 5)}{\text{Number of samples taken} * 5} * 100$$

concentration, which showed packages and gave good measurable results

Gel electrophoresis results: After adjusting all conditions and conducting diagnostic experiments on the RNA, the used primers showed different results and as shown in Fig. 3. The primer referred to as the symbol (*FMV*) in the form of (3) a band size 300 bp and this corresponds to what was reached (Elbeaino *et al.,* 2009) and 367 bp band was obtained from the use of the second primer referred to as (R1) in Fig. 3 and this corresponds to what was reached (Elbeaino *et al.,* 2009b). The primer referred to as the symbol (FLMAV-1) in the form of (4) a band size 352 bp and this corresponds to what was reached (Elbeaino *et al.,* 2006). The reliance on several diagnostic primers is one of the important steps that enhance the accuracy of virus diagnosis. Since the genetic variance of the *FMV* virus was low, the gene pathogen was the most important and fundamental factor in the formation of RNA by the flow of specific genes and encoded regions for RNA3, RNA4 have less genetic variation than RNA1, RNA2 through the changes of nucleotides and amino acids. The appearance of any type of RNA is caused by the expression of the species itself because it is primarily found in the genomic multiplex, and by the absence of the rest of the species by the process of RNA overlap, which inhibits the genes through

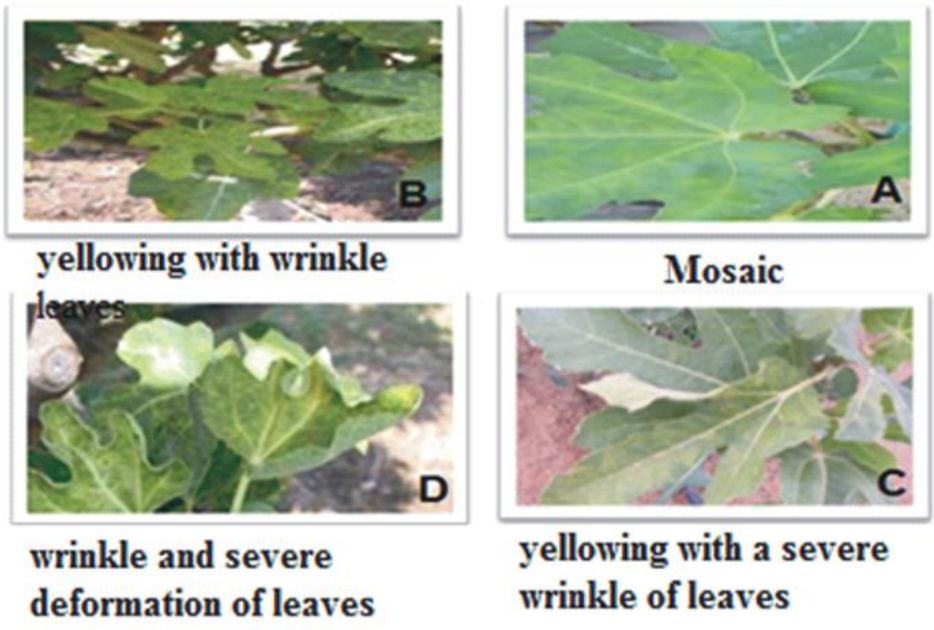

Fig. 2: Symptoms

the destruction of specific molecules in the mRNA because of exposure to changes prevented from expressing Such as the presence of the so-called silence genes and also the occurrence of the process of methylation and mutation the occurrence of changes in RNA, the reconstitution of the genomic RNA of the genome of different genetic isolates are similar. The suggestions in the presence of the four species are caused by functional or synthetic contraction of the encoded proteins as well as the thermal effect and environmental changes. Therefore, these studies of functional species are dependent on the direction of genetic mutations and reverse genetics.

Reducing the viral effects in Fig trees

Effect of different treatments on Percentage of Severity of infection in Fig plants

Effect of treatments used to reduce the percentage of intensity of infection in plants. The results of the statistical analysis showed that there was a high difference between the effect of the treatments in the percentage of the severity of the infection with the lowest percentage of the severity of the treatment of the moss *B. subtilis* + *S. platensis*, which amounted to 23.0% and the treatment of *G. lucidum* by 24.0% With the highest severity of 97.8% and the effect of the varieties there were significant differences between the yellow and black varieties were 25.371% and 26.269%, respectively. As for the bilateral interaction between the items and the transactions were observed significant differences gave the treatment *S. platensis*+*B. subtilis* for both varieties Black and yellow were the lowest proportion in the

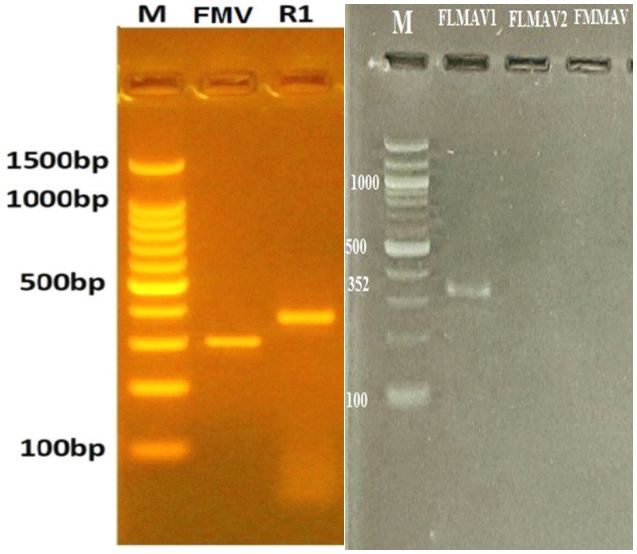

Fig. 3: Detection of *Fig Mosaic Virus (FMV)*

Fig. 4: Detection of *Fig Leave and RNA1 Mottle Associated Virus-1(FLMAV-1.*

severity of injury and reached 23.0% and gave the highest percentage In the severity of the injury to the yellow category reached 99.6%.

The difference in the severity of the infection is due to the difference in the type of treatment as the treatment of the bacteria caused the reduction in the severity of infection by stimulating the plant cells to resistance in the formation of proteins that affect the rate of multiplication of the virus and may also stimulate genes in the host on the production of anti-virus, Georg *et al.,* (1996), Alani *et al.,* (2011), It may also be due to the difference in the types of infection. This is similar to that of Abdul Jabbar

Table 2: Effect of treatment used in Percentage of Severity of infection.

Treatment varieties	Co	B.	Ga.	Sp.	B.+Ga.	B.+S.	Ga.+S.	Co.	Infected + Varieties
Yellow	0	19.6A	24.6AB	26.6BC	30.5B-D	23.0AB	24.6AB	96.0E	26.269A
Black	0	34.3B-D	23.3AB	26.6BC	35.5B-D	23.0AB	36.5B-D	99.6E	25.371A
Treatment effect	0	26.9B-C	24.0AB	26.6BC	33.1B-D	23.0AB	30.6B-D	97.8E	

et al., (2012) and Al-Fahad (2012) and Ayed and Al-Fahad (2018).

This can be explained by the fact that the studied cultivars are not highly resistant to the virus, but vary among themselves to respond to other factors to stimulate their resistance. This is demonstrated by the results of the research through the interaction between the *S. platensis* Moss with *B. subtilis* bacteria. This is in line with the findings of AL-Samarrai (2018) (B + Sp) gave the lowest mean of the control ratio of 8.61%. The closer the value of the treatment of one or more, the more likely that the genetic makeup is tolerable and the hybrid is more tolerable and more productive than normal This is similar to that of Al-fahad (2018). Studies indicated the possibility of using this type of bacteria to achieve the concept of self-resistant soils (Al-Fahad, 2006 and Mohsen, 2018). *Spirulina.* Spp. is characterized by its production of many effective and bio-active compounds in the biological system of various plant pathogens such as viruses, fungi and bacteria. The most important characteristic is that it is rich in the sources of proteins, vitamins and minerals, as well as other compounds such as amino acids, polyphenols, polysaccharides and dyes such as carotenoids and chlorophyll, The plant has different mechanisms to resist pathogenesis. This is in line with Ushrani *et al.*, (2015), Abbassy *et al.*, (2014), Ayed (2018) and Samarrai (2018). There is a significant effect of the transactions that contain Reishi mushrooms any notes. This may be due to the fact that it is a fungus that has the ability to fix *FMV* infection because it possesses compounds that are effective in stabilizing the viral infection, as the barriers prevent the virus from penetrating the plant cell and proved its ability to resist viral infection at high temperatures and Among the compounds that have proven to be effective in this fungus are polysaccharide polysaccharides such as GMX and this is in line with what (Mehta and Jandaik, 2012) have said.

Effect of different treatments on the percentage of chlorophyll for healthy figs infected with *FMV*

Results of statistical analysis in countries 2 showed that there was a difference in the chlorophyll ratio. There were significant differences between the treatment rates. The highest was the treatment of algae with bacteria (B + Sp) and 42.167. Spad followed by a 25.887 Spad control treatment. As for the effect of the varieties, there were no significant differences between the two varieties, reaching 36.8550 Spad for the yellow variety while the black variety reached 36.2396 Spad.

For the interaction of the infection with the treatment, the treatment of Spawel gave the highest percentage of chlorophyll 43,417 Spad followed by 42.9 Spad (B + Sp) treatment while the lowest chlorophyll ratio was 29,083

Table 3: Effect of treatment used in Chlorophyll Spad.

Treatment varieties	B.	Ga.	Sp.	B.+Ga.	B.+S.	Ga.+S.	Co.	Infected +varieties
Health Yellow	29.00J	34.60F-J	42.700A-D	42.033A-F	34.633F-J	45.533AB	35.067F-J	36.452A
Health Black	29.176J	36.667C-I	37.667B-I	36.967B-I	45.533A	37.333B-I	33.30G-J	36.396A
Infected Yellow	36.90B-I	39.76A-G	42.500A-E	41.367A-F	41.833A-F	39.90A-G	31.38IJ	37.285A
Infected Black	30.167IJ	39.20A-H	44.333AB	40.767A-G	43.967A-C	35.667F-J	30.167IJ	36.083A
Treatment varieties	B.	Ga.	Sp.	B.+Ga.	B.+S.	Ga.+S.	Co.	Infected +varieties
Infected	29.083G	35.633C-F	40.183A-C	39.500A-D	40.083A-C	41.433AB	34.183E-F	36.6708A
Health	33.533E-G	39.483A-D	43.417A	41.067AB	42.900AB	37.783B-E	30.773FG	36.4238A
Treatment varieties	B.	Ga.	Sp.	B.+Ga.	B.+S.	Ga.+S.	Co.	Infected +varieties
Yellow	32.95GH	41.233A-D	41.233B-D	42.267A-C	38.00C-F	43.683A	34.83GF	36.8550A
Black	29.667HI	34.983FG	38.433B-F	40.65C-E	43.150AB	40.65C-E	25.717I	36.2396A
Treatment effect	34.908B	31.308C	39.833A	41.458A	40.575A	42.167A	25.887D	

Spad.

As for the double interference of the injury and the varieties, the results showed that the infection was the highest rate of chlorophyll for the yellow category, which amounted to 37.285 Spad, or the lowest was the control treatment 30,083 Spad. The results showed significant differences and gave the treatment of mushrooms with moss the highest ratio of chlorophyll with 43.683 Spad for the yellow variety of the infected transactions followed by the treatment of B + Sp for the black class, which amounted to 43.150 Spad, while the lowest reached 30.77 Spad.

As for the Triple interference between the percentage of infections and the classes and treatments, the results showed that there was a difference between them and gave a Ga + Sp treatment for the treatment of the yellow color, and the treatment of B + Sp for the treatment of the black class had the same value of 45.533 Spad while the lowest value of chlorophyll gave it B treatment at 29.00 Spad.

The difference in chlorophyll ratios may be due to the effect of the known virus, which belongs to a group of viruses that affect the amount of green plastids. (Hemid, 2005), and the differences in chlorophyll ratios, especially the interaction between bacteria and moss, have been very effective. Systemic resistance of the plant by stimulating the proteins that affect the weakness of the virus and prevent its formation and thus the stimulation of resistance by the genes and with the overlap with algae, which contains Betain as a source of the element of nitrogen, which increases the amount of chlorophyll and prevent the analysis and when added leaves content of chlorophyll and thus an increase in the efficiency of absorption and an increase in the respiratory process and photosynthesis. This is in line with what the (Kuwada, 2006).

The chemical composition of algae indicates that the containment of large and micro nutrients and balancing them in plants improves growth in the vegetative and root groups (Kazem, 2012).

Most of the biochemical parameters used showed an incentive for resistance to systemic plants, which is characterized by low standards of infection and increased growth and outcome indicators.

The results of the biological experiments showed that treatment with S, F, and B, was the highest in reducing the incidence of tomato mosquitoes compared with control treatment and reducing negative effects in some growth indicators.

These results are also consistent with the findings of Thalig (2013) that the *B. subtilis* pollination of the other plants had a significant effect on the reduction of *BYMV* infection, which reduced the negative effects of the infection criteria.

In terms of varieties may be the genetic factor and genes of resistance against the virus, which the higher the higher the resistance status and thus reduce the amount of vaccine necessary for the occurrence of viral infection and increase the proportion of chlorophyll in plants and this is similar to what reached Albadry *et al*., (2006) and Mendo *et al.,* (2011).

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