

Biological control on Tomato Mosaic Virus (ToMV) by using some plant extracts

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ABSTRACT

This study was conducted in Department of Biology, College of Science, University of Anbar, to evaluate the efficiency of plant extracts; *Mirabilis jalapa*, *Eucalyptus*, *Camellia sinensis* to inhibit of tomato mosaic virus proliferation and use these extracts to stimulate the tomato plants resistance against the Tomato Mosaic virus when tested on indicator plants (*Datura stramonium*) under greenhouse conditions (Saran net shadow). Two concentrations used of each extract crude 100% and 50% concentration, and three replicates used for each treatment and concentration.

The results showed that the 50% concentration of both extracts was more efficient to inhibition the proliferation of the Tomato Mosaic virus in 1, 3 and 4 treatments. The results also showed that the *Mirabilis jalapa* extract was the most efficient to inhibition the virus in all treatments which gave 1.633 (77.660 %) and 2.380 (65.883%) lesion/cm² in concentration 50 % and crude respectively in the treatment 1 and gave 1.966 (67.786%), 1.136 (82.576%) lesion/cm² in concentration 50% and crude respectively in the treatment 2 and gave 1.886 (74.057%), 2.470 (69.201%) lesion/cm² in concentration 50% and crude respectively in the treatment 3 while the treatment 4 gave number of lesions 1.970 (74.827%), 2.353 (70.177%) lesion/ cm² in concentration 50% and crude respectively compared with the distilled water (control treatment) and with the other plant extracts in our study. *Eucalyptus* extract was the second level in its efficiency to inhibition of tomato mosaic virus by reduction the number of lesions per square centimeter of the leaf followed by the extract of *Camellia sinensis* which gave the third level in its ability to inhibit the virus.

Keywords: Tomato, Mosaic, Virus, (ToMV), *Datura*, Biological control, *Mirabilis jalapa*, *Eucalyptus*, *Camellia sinensis*

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INTRODUCTION

The plant viruses have high importance to farmers and researchers because of high economic losses they cause in different crops like fruit, vegetable, cereal and legume (Waterworth and Hadidi, 1998). Tomato plant (*Lycopersicon esculentum*) is a dicots and flowering plant and classified under solanaceae and have food, economic importance (Alubaidi and Almaeini, 2018), but this important crop infected with several virus diseases like tomato mosaic virus (ToMV) which destruct this crops in many countries (Daniela *et al.*, 2009). This virus can continue in its proliferation in infected plants and can to infect new plants and cause destruction of the green plastids inside the cells and causing a decrease in the amount of chlorophyll in the plants as one of the viruses causing the symptoms of mosaic (El- Afifi and others, 2004). The plant which infected with the virus has wrinkle leaves and wraps their blade down with dwarfing plant (Athab, 2009). Due to the absence of chemical pesticides to control on the viruses as well as their danger on environment, many attempts used to control on viruses by plant extracts (Azzawi, 2002), because the plants are important sources of antimicrobial compounds, plants extracts have a great effective against a wide range of pathogens (Srivastava *et al.*, 2010). Researchers have been tested many inhibitors derived from various plants to control on plant viral diseases (Ito *et al.*, 1992; Kubo *et al.*, 1990), many medicinal plant have potent antiviral activity and some of them used to treat animals and people who suffer from viral infection (Hudson, 1990).

The aims of our research are:

- 1- Using plant extracts to inhibit the Tomato Mosaic Virus multiplication.
- 2- Using plant extracts to induce the resistance in plant against Tomato Mosaic Virus.

MATERIALS AND METHODS

Sterilization of soil and peat moss:

The soil and peat moss which used in the agriculture with quantity 1: 2 were sterilized using an autoclave at 121^o C, 15 bars for 45 minute, and left a week before using in agriculture.

Sterilization of pots:

The plant pots were sterilized with hypochlorite 6 % for 15 minute, then washed with water to removing the remaining hypochlorite, then used for agriculture.

Preparation of soil and plants cultivation:

Tomato seeds were grown in pots with 12 cm in diameter and 11 cm in height, which packed with sterile soil and peat moss, then putted in green house (1.5 meter in height, 4 meter in length and 3 meter in width). The temperature between 28 – 30 ° C. Used commercial fertilizer NPK at concentration 3 g /L of water. Insecticide aerotox sniper was used with concentration 10 % every week as a minimum.

The plants of the study:

The tomato seeds *lycopersicon esculantum* were collected from markets in Al- Anbar from Argeto Company, and *Datura* seeds *Datura stramonium* were obtained from general organization for seeds certification, College of Agriculture, University of Baghdad, Iraq.

The plant extracts preparation:

Water extracts was prepared by taking 10 g of dried leaves powder of *Mirabilis jalapa*, *Eucalyptus*, *Camellia sinensis* and placed in 500 ml glass flask which containing 200 ml distilled water, then mixed with a magnetic stirrer for 15 minute, The leaky was filtered through three layers of gauze, then centrifugation for 15 minute to separate the small molecules and obtain a clear solution, then the volume completed to 100 ml with distilled water. The final solution was diluted to half to get concentration 50 % of extracts.

Isolation of tomato mosaic virus (ToMV):

Tomato mosaic virus isolated from *Datura* plants which infected with the virus, then took the leaky of the *Datura*

and used in inoculation of healthy tomato plant, when the symptoms of mosaic appeared took the leaky of tomato and treated at a temperature between 90 – 92 ° C in water bath for 10 minute (Zaitlin and Israel, 1975), which used to inoculum the leaves of *Datura* and wait for 7 days until the symptoms appear (local lesions with brown color), then took one local lesion to inoculum healthy tomato plant, after the symptoms appeared on the plants they are using as the first source of ToMV inoculum for later experiments.

Preparation of tomato mosaic virus inoculum:

1 gm of virus-infected leaves crush with 5 ml of distilled water (Alsaedy, 2004). The leaky was separated through 3 layers of gauze and then centrifugated and kept in container in frozen for later experiments.

Mechanical inoculation process:

Carborundum 400 mesh sprayed by dispenser on the leaves, the leaf supported by the left hand, then the finger of the right hand dipped in the inoculum and wiped the leaf with light pressure, then washed the leaf with water after 20 seconds of inoculation (Alsaedy, 2004).

Calculation of insulated leaves areas (cm²):

Draw infected leaves (which have local lesions) on the charts, and then account the whole squares according the equation:

Leave area (cm²) = No. of complete squares + No. of incomplet squares / 2 (Felejah, 1987).

The experiments:

1- Effect of mixing the viral inoculum with the Plant Extract on the proliferation of tomato Mosaic virus.

This experiment was conducted to study the effect of mixing of the plant extract with the viral inoculum on the infected *Datura* plants. The sample of treatment in this experiment included mixing the crude or 50 % of plant extract with the viral inoculum with ratio 1: 1. (The control sample consisted of mixing the viral inoculum with distilled water by 1: 1), then leaving the mixture for one hour, and then treated *Datura* leaves with this mixture for each concentration or treatment. The results were taken after 8 days of inoculation (local lesions on *Datura* leaves). The treatments as follows:

- 1- 1 ml virus inoculum + 1 ml crud extract of *Eucalyptus*
- 2- 1 ml virus inoculum + 1 ml 50% extract of *Eucalyptus*
- 3- 1 ml virus inoculum + 1 ml crud extract of *Mirabilis jalapa*
- 4- 1 ml virus inoculum + 1 ml 50% extract of *Mirabilis jalapa*
- 5- 1 ml virus inoculum + 1 ml crud extract of *Camellia sinensis*
- 6- 1 ml virus inoculum + 1 ml 50% extract of *Camellia sinensis*
- 7- 1 ml virus inoculum + 1 ml distilled water (control)

2- Effect of spraying healthy tomato plants with plant extract on tomato mosaic virus multiplication:

Healthy and homogenous tomato plants with age of the fourth leaf were selected and sprayed with the plant extracts, then left after spraying for 24 hours. After that they were inoculated with the viral inoculum and left for a week until the symptoms of mosaics appeared. Then *Datura* plant were treated with leaky of infected tomato leaves and left for 8 days until the symptoms appeared (local lesions). Then calculated the number of lesions in cm² of *Datura* leaves.

The treatments as following:

- 1- Spraying tomato plant with crud extract of *Eucalyptus*
- 2- Spraying tomato plant with 50% extract of *Eucalyptus*
- 3- Spraying tomato plant with crud extract of *Mirabilis jalapa*
- 4- Spraying tomato plant with 50% extract of *Mirabilis jalapa*
- 5- Spraying tomato plant with crud extract of *Camellia sinensis*
- 6- Spraying tomato plant with 50 % extract of *Camellia sinensis*
- 7- Spraying tomato plant with distilled water (control treatment)

3- Effect of immersion of tomato plant roots in the plant extracts on tomato mosaic virus multiplication:

Five plants were extracted from the soil with fourth leaf age, their roots were washed with running water and immersion in plant extracts (in each extract and concentration), immersion on plant in the distilled water as a control and left immersed for 24 hours, all treatments were transferred to the distilled water, then inoculated one leaf per plant with virus inoculum and left for 7 days. After that *Datura* leaves inoculated with the leaky of inoculation tomato plants and left until the symptoms appeared on *Datura* plant. Then calculated leaves area and number of local lesions for each cm² from leaves.

The treatments as following:

- 1- Immersion of tomato roots in crud extract of *Eucalyptus*
 - 2- Immersion of tomato roots in 50% extract of *Eucalyptus*
 - 3- Immersion of tomato roots in crud extract of *Mirabilis jalapa*
 - 4- Immersion of tomato roots in 50% extract of *Mirabilis jalapa*
 - 5- Immersion of tomato roots in crud extract of *Camellia sinensis*
 - 6- Immersion of tomato roots in 50% extract of *Camellia sinensis*
 - 7- Immersion of tomato roots in distilled water (control)
- 4- Effect of immersion of *Datura* roots in the plant extracts on resistance stimulating in these plants (No. of lesions / cm²).**

Homogenous *Datura* seedlings with their roots, with fifth leaf age (washed well with running water) were immersed in the plant extracts They were left for 24 hours (The control treatment was to immerse the roots of *Datura* in the distilled water), then transferred to the distilled water and inoculated by the virus by three replicates for each treatment and concentration and then left after the inoculation for 8 days until the symptoms were appeared. Then calculated the areas of *Datura* leaves and extracted the number of lesions in cm² of leaf. The treatments as following:

- 1- Immersion of *Datura* roots in crud extract of *Eucalyptus*
- 2- Immersion of *Datura* roots in 50% extract of *Eucalyptus*
- 3- Immersion of *Datura* roots in crud extract of *Mirabilis jalapa*
- 4- Immersion of *Datura* roots in 50% extract of *Mirabilis jalapa*
- 5- Immersion of *Datura* roots in crud extract of *Camellia sinensis*

- 6- Immersion of *Datura* roots in 50% extract of *Camelia sinensis*
- 7- Immersion of *Datura* plant in distilled water (control treatment)

Statistical analysis:

We used three replicates for each treatment and concentration, the results were analyzed according to the statistical program, SPSS by using complete randomized design CRD with significant level 0.05 ($P \leq 0.05$).

THE RESULTS

1- Results of mixture the viral inoculum with Plant Extracts in Tomato mosaic virus multiplication by Number of lesions / cm² in *Datura* plant.

The results in the table 1 show the number of lesions in cm² of leaf according to treat the *Datura* leaves by mixture of plants extracts with tomato mosaic virus and

comparing that with the control (mixture of tomato mosaic virus with distilled water).

The concentration of 50% of *Mirabilis jalapa* extract gave a high ratio in virus inhibition which gave the number of lesions 1.633 lesion / cm² while the crud extract gave 2.380 lesion / cm², both concentration gave significant differences with the control which gave number of lesions 7.310 lesion / cm². While the concentration of 50% of *Eucalyptus* extract gave 2.386 lesion / cm² with the significant differences with the crud extract of *Eucalyptus* which gave 3.373 lesion / cm² and these two concentration were gave significant differences with the control which gave 7.310 lesion / cm² of *Datura* leaf (indicator plant), *Camelia sinensis* extract was gave the inhibition of virus and gave number of lesion reach to 3.686 and 4.696 lesion / cm² in 5% and crud extract respectively comparing with the control which gave 7.310 lesion / cm².

Table (1) Number of lesions which resulting from mixture plants extracts with viral inoculum in multiplication of tomato mosaic virus (No. of lesion / cm²)

extracts	Number of lesions / cm ²			The average
	Con.	Crud	50%	
<i>Mirabilis</i>		2.380 ±0.160 A	1.633±0.083 E	1.906±0.386
<i>Eucalyptus</i>		3.373±0.118 B	2.386±0.302 G	2.880±0.578
<i>Camelia</i>		4.696±0.228 C	3.686±0.516 F	4.191±0.658
Water		6.976±0.180 D	7.310±0.278 D	7.143±0.278

The numbers of similar letters are not significantly different from each other according to the test of the least significant difference L.S.D and the level of significance $P \leq 0.05$

2- Effect of spraying healthy tomato plants with plant extracts in multiplication of the tomato Mosaic virus by No. of lesions / cm².

he results of the statistical analysis which mentioned in Table (2) showed that the crud extract of *Mirabilis jalapa* was gave a highest ratio in the inhibition of the virus multiplication by giving a number 1,136 lesion / cm² comparing with concentration of 50%, which gave a

number of lesions 1.966 lesion/ cm² both concentrations were gave significantly differences With treatment of the control which gave a number of lesions 6.520 lesion / cm². The results also showed that the crude extract of *Eucalyptus* was gave 2,066 lesion / cm² with not significant differences with a concentration of 50% which gave 2.210 lesions / cm² and compared with the control treatment which gave 6.520 lesions / cm². While the crud extract of *Camelia sinensis* gave No. of lesions 2,300 lesion / cm² with a significant difference with a concentration of 50% which gave 2.906 lesion / cm² while control treatment showed No. of lesions 6.520 lesion / cm².

Table (2) Effect of spraying of healthy tomato plants with the plant extracts in tomato mosaic virus multiplication by No. of lesions / cm² in *Datura* plants (indicator plant).

extracts	Number of lesion/ cm ²			The average
	Con.	crud	50%	
<i>Mirabilis</i>		1.136±0.148A	1.966±0.051E	1.551±0.465
<i>Eucalyptus</i>		2.066±0.057B	2.210±0.110B	2.138±0.111
<i>Camelia</i>		2.300±0.100C	2.906±0.105F	2.603±0.344
Water		6.520±0.475D	6.103±0.115D	6.311±0.384

The numbers of similar letters are not significantly different from each other according to the test of the least significant difference L.S.D and the level of significance $p \leq 0.05$.

3- Effect of immersion the roots of tomato plants in plant extracts in stimulating the resistance against tomato mosaic virus by number of lesion / cm² (in indicator plant).

The readings listed in Table (3) indicate that the immersion of tomato plants roots in the plant extract of *Mirabilis jalapa* at a concentration 50% lead to stimulate the resistance against the tomato mosaic virus, which resulted in a decrease in the number of lesion/cm² of leaf, which gave 1,886 lesion / cm², while the crude extract gave the number of lesion 2,470 lesion / cm². Both concentrations had significantly differences with the

control treatment, which gave the number of lesions amounted to 8.020 lesion / cm².

The results in the same table indicate that the concentration 50% of *Eucalyptus* extract gave the highest resistance against the virus represented by the decrease in the number of lesion in *Datura* plant (indicator plant) which gave 2.070 lesion / cm² with significantly different from the crude concentration, which gave a total of 2,830 lesion / cm² and compared with the control treatment. As well as 50% concentration of *Camelia sinensis* extract gave a highest ratio than the crude concentration in reducing the number of lesions which gave 3.103 lesion / cm². While the crude extract of *Camelia snensis* gave the number of lesions 3,890 lesion / cm² and the two concentrations gave significantly differences with the control, which gave a number of lesion 7.2700 lesions / cm².

Table (3) Effect of immersion of tomato plants root in plant extracts for stimulating the resistance against tomato mosaic virus by number of lesion / cm² in indicator plant (*Datura*).

Extract	Number of lesion/ cm ²			Average
	Con.	Crud	50%	
<i>Mirabilis</i>		2.470±0.308A	1.886±0.100H	2.178±0.379
<i>Eucalyptus</i>		2.380±0.196B	2.070±0.112G	2.450±0.440
<i>Camelia</i>		3.890±0.100C	3.103±0.095F	3.496±0.439
Water		8.020±0.090D	7.2700±0.376E	7.645±0.478

The numbers of similar letters are not significantly different from each other according to the test of the least significant difference L.S.D and the level of significance $p \leq 0.05$.

4- Effect of immersion of *Datura* plants roots in the plant extracts for stimulating the resistance in these plants against tomato mosaic virus by number of lesions/ cm².

The statistical analysis of the results listed in Table (4) indicates that the immersion of the *Datura* plants roots in the *Mirabilis jalapa* extract at a concentration 50% reduced the number of lesions per centimeter from leaf area, which gave number of lesion 1.970 lesion / cm² while the crud extract of *Mirabilis jalapa* gave 2,350 lesion / cm² And the control treatment giving a total of 7,890 lesion / cm².

Table (4) Effect of immersion of *Datura* plants roots in plant extracts in stimulating the resistance in these plants against tomato mosaic virus by accounted lesion / cm².

extract	Number of lesion/cm ²			The average
	Con.	crud	50%	
<i>Mirabilis</i>		2.353±0.125A	1.970±0.052G	2.161±0.226
<i>Eucalyptus</i>		2.916±0.011B	2.026±0.219F	2.471±0.506
<i>Camelia</i>		3.250±0.219C	2.760±0.196E	3.005±0.326
Water		7.890±0.091D	7.826±0.111D	7.858±0.097

The numbers of similar letters are not significantly different from each other according to the test of the least significant difference L.S.D and the level of significance $p \leq 0.05$.

THE DISCUSSION

This study included test of efficacy of plant extracts of *Mirabilis jalapa*, *Eucalyptus* and *Camelia sinensis* in reducing the multiplication of tomato mosaic virus by testing its effectiveness in inhibiting by interaction of the virus when mixed with it (extracts) and treatment of plants with them, whether tomato or *Datura*. The results of the tables (1, 2, 3 and 4) showed a very clear reduction in the number of lesions caused by the virus compared to the control treatment. *Mirabilis jalapa* extract had the first level among the extracts used in this research at the inhibition of proliferation of the virus, where it used in the four experiments, showed the lowest number of lesions from other plant extracts, because there is a special protein in the plant called *Mirabilis* antiviral protein (MAP) and this protein is anti-virus X and Y in the potato has been treated with this plant before 24 hours of viral inoculation and showed inhibition of the

The results in the same table indicate that 50% of eucalyptus extract gave higher ratio than the crude extract, which led to a decrease in the number of lesions to 2.026 lesion / cm², while the treatment of the crude extract gave the number of lesion 2.916 lesions / cm². Both concentrations were gave significantly differences with the control treatment (water), reach to 7.890 lesion/cm².

Finally, the results of the table also showed that 50% of the *Camelia sinensis* extracts exceeded the crude concentration. The crude gave a total of 3,250 lesion / cm², while the 50% gave 2,760 lesions / cm², while a control treatment gave a number of lesion per centimeter of the leaf 7,890 lesion / cm².

virus by 100% has been purified this protein and found that it has the same antiviral effect in the case of being crud or purified (Jorge *et al.* 1999). While *Eucalyptus* took the second level in its strength to discourage the virus, our results agree with the results of AL- Janabi (1984) when he found that the *Eucalyptus* leaves protect the tobacco plants from tobacco mosaic virus for 30 days after spraying on plants. *Eucalyptus* extract may also have a high capacity of cyanogenic capacity due to the concentration of toxic glycosides and its toxicity increase when growth the plants in the hot environment (Gleadow and wordrow, 2002). *Camelia* extract was had the third level in inhibition the tomato mosaic virus, Bawden (1954) mention that *Camelia snensis* extract contains tannins These substances effects on the effectiveness of the virus outside the living tissue.

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