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# Enhancement of Systemic Acquired Resistance in *Lycopersicon esculentum* L. against Tobacco Mosaic Virus by Salicylic Acid

BASIM HASAN KHEDHAIR
Department of Animal Resources
College of Agriculture, University of Sumer
Thi-Qar, Iraq
Basimhasan8@gmail.com

**Abstract:**

*This study was conducted to evaluate the efficacy of foliar sprays by salicylic acid which induces systemic acquired resistance (SAR) in plant and determine the effect on Tobacco mosaic virus in tomato under greenhouse conditions. The study includes: Biological evaluation of virus by local lesion assay and serological evaluation by DAS-ELISA. Salicylic acid foliar sprays application showed significant differences in Percentage of inhibition to number of local lesions 66% and reduction of the local lesion size was **59** % on N. glutinosa compared with control application. Serologically, DAS-ELISA values under 405 nm also showed significant differences 0.049 compared with the positive control 0.096. These finding suggested efficiency salicylic acid in enhancement systemic acquired resistance in tomato plants against TMV under greenhouse conditions.*

**Key words:** Systemic acquired resistance, tobacco mosaic virus, salicylic acid, DAS-ELISA, *N. glutinosa*

## 1. INTRODUCTION

Tomato crop has many constrains which faced it is cultivation in different parts of the world. Among those constrains was

attacked by various diseases such as fungal and viral diseases, Recently virus diseases of tomato were considered the most important disease which infected the crop particularly tobamoviruses, tobacco mosaic virus (TMV), Viral diseases have become a menace to the farmers as well as to the scientist who are involved in the production of seeds and seeds quality (Madhusudhan et al., 2008). Systemic acquired resistance (SAR) is a broad-spectrum defense system present in plants, which was realized as early as by Ray in 1901. Several synthetic chemicals have been shown to be inducers of SAR response in variety of crop-pathogen systems (Mandal et al., 2008 cited in Ray, 1901). In recent years, salicylic acid (SA) was used in inducing systemic acquired resistance against several pathogens includes fungi, bacteria and nematodes and due to the shortage of information in studies related to using SA in inducing resistance against the viruses and in order to extend our knowledge on the effect of this agent as a chemical inducer against TMV in tomato plants this study was carried out:-

1- Evaluate the efficiency of foliar sprays for salicylic acid by detection of inactivation of virus replication by Bioassay.

2- Evaluate the efficiency of foliar sprays for salicylic acid by detection of inactivation of virus replication by Serological assay (DAS-ELISA)

## 2. MATERIALS AND METHODS

### 2-1. Sampling

Infected plants with suspected infected with tobacco mosaic virus were collected from one of greenhouses at Baghdad for season 2015.

## 2-2. Detection of Virus

Depending of artificial inoculation, symptoms occurred on indicator plants and immunostrip ELISA serological test, the virus was identified.

### 2-2-1.The Indicator Plants

In regard to virus detection used plants as show (table 1)

**Table 1. The indicator plants used in detect of virus.**

| English name | Scientific name | Symptoms |
|---|---|---|
| **Tobacco** | ***Nicotiana tabacum var Turkish L.*** | Systemic mosaic |
| **Tobacco** | ***Nicotiana glutinosa*** | Necrotic local lesions |
| **Tomato** | ***Lycopersicon esculentum Mill.*** | Systemic mosaic |
| **Chenopodium** | ***Chenopodium amaranticolor Reyn and Coste*** | Chlorosis local lesions |
| **Cucumber** | ***Cucumis sativus L.*** | Symptomless |
| **Cowpea** | ***Vigna unguiculata L.*** | Symptomless |

### 2-2-2. Immunostrip ELISA

ELISA was performed by using viral antiserum for TMV as flash kits (provided from Agdia biofords, USA) according to the procedure of recommended by the company as follows:

**1-** A part of a leaf plant sample was cut and placed into an extraction bag which includes extraction buffer (PBS).

**2**- The sample was homogenized with a hand homogenizer on flat surface.

**3**-The tip of strip was soaked for 0.5 cm in the extract then observed format of colored bands.

**4**-The results were recorded after 3-5 min.

**5**-The above steps were repeated with extract of tomato healthy plants.

## 2-3. Mechanical Inoculation:

The standard virus inoculum was prepared by using the tomato leaves showing mosaic symptoms. The leaves (1g) were homogenized in 5ml of the phosphate buffer pH 7.0 in a pre-chilled pestle and mortar. The sap extract was passed through

muslin cloth then, the filtrate was used as a source of inoculum. The cotton swab was dipped in the virus inoculum and rubbed over previously dusted with an abrasive powder (carborundum 600 meshes).. After inoculation the leaves were washed with water. phosphate buffer solution consist of (1.362 gm KH2PO4 in 1000 ml distilled water ,1.781g $No_2Hpho_4$. 2 $H_2O_2$ in 1000 ml water, 51 ml of the $Na_2pho_4$ solution mixed with 49 ml KH2Po4 solution gives solution pH 7.0 (Al-Ani and Rathi. 1984). The inoculated plants were kept at the greenhouse and watched daily to record any symptom which possibly appeared on the plants.

### 2-4. Propagative Plants

Tomato is unsuitable for propagating or maintaining the virus because it gives low yields. Preparations contain more impurity, and it can selectively change the character of isolates. For propagating the virus, plants *Nicotiana tabacum* var. Turkish L were used as a propagate host for virus. Also *N. tabaccium .var samsum* was used as a propagate host.

### 2-5.Preparation of Pure Isolate

The *N. glutinosa* plants were inoculated with a diluted crud sap of infected tomato leave, after 2day the local lesions occurred on the leave surface. Sap of single local lesion was extracted and used as inoculum for mechanical inoculation on *N. tabacum* var. Turkish. A serious of inoculation on a local lesion host alternating with a systemic host was done and pure isolate from the virus was obtained (Al-Ani and Rathi. 1984).

## 3. PREPARATION OF SA

Salicylic acid (MW 132) of Sigma Aldrich was used dissolved 2 mM SA in 1 L of distilled water. Foliar applications were applied after 20 days from transplanting.

## 4. PLASTIC HOUSE EXPERIMENT

### 4-1. Agricultural Processes

Prepare one plastic house at dimension (52×9) m then; the agricultural processes were performed including tillage, leveling, installing drip irrigation system, and fertilization with organic manure before planting. The plastic house was divided into 4 lines and between one plant and another 40 cm, leaving space between the treatment and another 80 cm.

### 4-2. Distribution of the Treatments and Experimental Design.

The plastic house was divided into 3 replicates, each replicate includes all treatments then, treatments were arranged randomly according to RCBD. The means were tested according to LSD (P=0.05). Data was analyzed by GenStat discovery 12th Edition with factorial design two ways with blocking (Elsahookie and Wahaib, 1990).

### 4-3. Foliar Sprays Treatments

Foliar applications were applied after 20 days from transplanting.

The experiment included 4 treatments as follows:

1- Foliar sprays with SA application at concentration 2 mM/L 4 days before the mechanical inoculation.
2- Foliar application with distilled water as a control treatment.
3- Foliar sprays with SA at concentration 2 mM/L without mechanical inoculation.
4- Foliar sprays with distilled water without the mechanical inoculation.

## 5. BIOLOGICAL EVALUATION

The effect of SA was quantified by local lesions assay on *N. glutinosa*. The first five leaves of *N, glutinosa* plants were inoculated with the infected tomato crude sap extracted from treated plants with SA 2 mM/L, in addition to distilled as control for each treatment in the plastic house experiment. Two leaves of each plant were rubbed with the infected tomato crud sap. Number and size of the local lesions were counted to each inoculated leaf explants after incubation for 7 days. The effect of SA on TMV was quantified by number and size local lesions which form on *N. glutinosa*. The percentage inhibition of local lesions on the inoculated leaves was calculated by using the following formula (Zhang et al., 2010):

$$I= (C- T) \times 100/ C$$

Where, I = percent inhibition of local lesion formation over control; C = Number of local lesions in control; T= Number of local lesions in plants treated with SA.

## 6. SEROLOGICAL EVALUATION.

The virus concentration in the inducer-treated as well as the control plants was quantified by using DAS-ELISA (supplied by Agdia biofords ,India). After (7, 14, 21) day, three plants from each replicate were harvested for detecting the virus concentration. The leaves (1g) were harvested and crushed with 1ml of the tissue extraction buffer in a mortar and pestle then, passed through 2 layers of muslin clothe, the extract was subjected to DAS-ELISA. Whereas DAS- ELISA procedure includes as follows:

1-preparing humid box: A humid box was prepared by lining an airtight container with a wet paper towel. Test wells (micro ELISA plate) were kept in a humid box during a

required incubation will help prevent samples from evaporating and dryness.

2- Preparing capture antibody: One hundred µl capture antibody anti TMV IgG was added to 10 ml carbonate coating buffer for preparing capture antibody solution. The prepared capture antibody solution was mixed and used immediately. Then, by micropipette 100 µl of the prepared capture antibody solution were added into each well.

3- The plate was placed in a humid box and incubated at overnight in the refrigerator (4C°).

4- The wells emptied into waste container then, the Test wells were filled completely with 1x PBST, and quickly emptied them again. This process was repeated 2 more times.

5- Grind and dilute samples: TMV infected tomato leaves were grinded in sample extraction buffer by mortar and pestle at a 1: 10 ratio (leaf weight in g: buffer volume in ml). 100 µl of diluted sample extraction were used per test wells.

6- Dispense samples: 100µl of prepared sample dispensed into sample wells and set inside the humid box then, incubated for 2 hours at 37C° in the incubator.

7- Prepare enzyme conjugate: For preparing enzyme conjugate solution, IgG- conjugated with alkaline phosphatase must be diluted with Enzyme conjugated buffer before use.100 µl of the concentrated enzyme conjugate were added to 10 ml Enzyme conjugated buffer then, when the sample incubation is complete the plate was washed by a quick flipping motion to dump the wells into a sink without mixing the contents. All the wells were completely filled with 1x PBST and then quickly empty them again repeated 7 times.

8- Adding IgG- conjugated with enzyme: 100 µl of prepared IgG enzyme conjugate were dispensed per well then, incubated the plate in humid box for 3 hours at 37C°.

9- Preparing polyvinyl pyrolidone (PNP) solution: Each PNP tablet was dissolved to make 5 ml of PNP solution, at a concentration of 1mg / ml, about 15 min. before the end of the

above incubation step. Then, without touching the tablets, the PNP tablets were added to the buffer and without touch the PNP tablets or expose the PNP solution to strong light because of Light or contamination could cause background color in negative wells. Then, the plate was washed 8 times with 1x PBST.

10- Adding PNP substrate: 100 µl of PNP substrate were dispensed into each test wells. Then, the plate was incubated for 60 min at 37C°. plates should be protected from direct or intense light.

11- Evaluating results: The wells were examined by a plate reader (BioTek ELX800) at 405 nm according. Wells in which yellow color develops indicate positive reaction. Whereas wells in which there is no significant yellow color developed indicate negative result.

## 7. RESULTS AND DISCUSSIONS

### 7-1. The local lesion assay

The results of bioassay experiment showed that the number and size of local lesions occurred on SA spray treated plants were reduced significantly compared with number and size of local lesions formed on control plants (untreated).

**Table 2. Effect of foliar spray of on TMV Infection on Tomato as measured on a local lesions formation on *Nicotiana glutinosa*.**

| Treatment | No. of local lesions/ leaf | Size of local lesions (cm) | inhibition of number local lesions % | Reduction in local lesion size % |
|---|---|---|---|---|
| SA<br>2 mM/L | 12.32<br>±1.77° | 0.41<br>±0.03° | 66 | 59 |
| control | 36.24<br>±1.04° | 0.52<br>±0.03° | 0.00 | 0.00 |
| LSD<br>P = 0.05 | 3.64 | 0.09 | 9.4 | 7.5 |

*Each value represents the mean of three replicates.

## 7-2. Serological Evaluation

The DAS-ELISA values for SA treated tomato seedlings showed significant reduction in the viral concentration for the weeks mean compared with positive control plants (table 3).

**Table 3. DAS-ELISA values for foliar spray treatments with SA**

| Treatment | Mean of ELISA values<br>Date of plant sampling | | | |
|---|---|---|---|---|
| | 1week | 2week | 3week | Mean |
| SA | 0.032 | 0.039 | 0.078 | 0.049 |
| Positive control | 0.039 | 0.065 | 0.173 | 0.096 |
| Negative control | 0.012 | 0.011 | 0.013 | 0.012 |
| LSD P=0.05 | 0.005 | 0.004 | 0.006 | 0.004 |

*Absorbance at 405 nm.

These finding suggested efficiency salicylic acid in enhancement systemic acquired resistance in tomato plants against TMV under greenhouse conditions.

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