

## Evaluation of TMV resistance inducers in tomato

Ramiro Hernández-Santiago<sup>1§</sup>  
Mateo Vargas-Hernández<sup>1</sup>  
Erika Janet Zamora-Macorra<sup>2</sup>

<sup>1</sup>Department of Agricultural Parasitology-Chapingo Autonomous University. Mexico-Texcoco Highway km 38.5, Texcoco, State of Mexico, Mexico. CP. 35230. Tel. 595 9521608. (rhsmpv16@gmail.com; mateo-vargas@hotmail.com). <sup>2</sup>Department of Agricultural Preparatory-Chapingo Autonomous University. Mexico-Texcoco Highway km 38.5, Texcoco, State of Mexico, Mexico. CP. 56230. Tel. 595 9521677. (erikazam@gmail.com).

§Corresponding author: rhsmpv16@gmail.com.

### Abstract

Applications of resistance inductors were made: Messenger gold<sup>®</sup>, Virus Stop<sup>®</sup>, Actigard<sup>®</sup>, Virablock<sup>®</sup>, Kendal<sup>®</sup>, potassium phosphite, Stymulus<sup>®</sup> Maxx, *Bacillus subtilis* and MC Cream<sup>®</sup> in 2018, individually and in combination in sequences, to evaluate its effect on viral concentration (optical density), growth, root length, severity, total dry weight, number and weight of fruits in the tomato crop (*Solanum lycopersicum* L.) infected with *Tobacco mosaic virus* (TMV). The sequence of the Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup> inductors reduced the concentration of TMV at 38 days after inoculation, determined by DAS-ELISA. Virablock<sup>®</sup> spray obtained the highest average height. Plants treated with the sequence Virus Stop<sup>®</sup> + Virus Stop<sup>®</sup> + potassium phosphite + potassium phosphite obtained the lowest average severity. Plants sprinkled with Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup> recorded the longest root length, highest total dry weight and the second highest number and average weight of fruits that showed no symptoms of TMV in fruits.

**Keywords:** Elisa, resistance inductors, solanaceae.

Reception date: January 2020

Acceptance date: March 2020

## Introduction

Tomato (jitomate) (*Solanum lycopersicum* L.) is economically one of the most important horticultural crops worldwide; however, diseases caused by viruses can cause serious damage and economic losses, between 5 to 90% (Hanssen *et al.*, 2010; Sikora, 2011). In 2017, 5 226 hectares were harvested with 3 469 707 tons of production in Mexico (SIAP, 2018).

World production in 2016 was 177 042 359 t, China, the main producer produced 56 308 914 t (31.81%) (FAOSTAT, 2018). In Mexico, the production of red tomato grew at an average annual rate of 3.6% between 2007 and 2017, it was the main tomato exporter in terms of volume 1.68 million tons in 2018 and supplied 84.9% of the volume of imported fresh tomato for the United States of America. On the other hand, the per capita consumption of tomatoes in Mexico is 16 kg year<sup>-1</sup> and the world average per capita was 19 kg (FIRA, 2019).

The diseases of viral origin in tomato are very important in the country due to the losses that can cause in the production, as well as to the high cost that its handling implies. In many producing areas, the impact of these diseases has been devastating and in extreme cases the cultivated area has diminished considerably (Bautista *et al.*, 2010). The tobacco mosaic virus is distributed in virtually all tomato producing areas in Mexico (Ley and García, 1998).

The induction of resistance against pathogens, such as acquired systemic resistance (RSA) and induced systemic resistance (RSI), has been observed by various elicitors against fungi, nematodes, parasitic plants and viruses (Vallad and Goodman, 2004). There are a variety of molecules that participate in resistance to diseases that are promoted with the exogenous application of elicitors such as salicylic acid (AS), ethylene (E), acibenzolar S-methyl and oligosaccharides that have been used in various crops in the field and greenhouse (Schreiber and Desveaux, 2008). Vallad and Goodman (2004); Pieterse and Van Wees (2015) found some characteristics to differentiate, acquired systemic resistance (RSA) and induced systemic resistance (RSI).

The (RSA) is induced by biotic or abiotic elicitors, induces PR proteins, uses signaling pathways that can involve salicylic acid (AS) and its signal travels systemically to distal sites where the infection occurred. The (RSI) is potentiated by growth promoting rhizogenic bacteria (BPCV), does not involve the synthesis of PR proteins and the signaling route is carried out through jasmonates and ethylene. The term elicitor is commonly used for compounds that stimulate any type of defense in plants (Ebel and Cosio, 1994).

Eventually, inducing defense responses can lead to improved resistance (Thakur and Singh, 2012). In previous research in tomato plants treated with Acibenzolar S-methyl (ASM) and acetylsalicylic acid (ASA), they presented the lowest levels of incidence and severity of virosis at the different evaluation dates under greenhouse conditions (Pérez *et al.*, 2017). In zucchini it was found that the application of *Bacillus subtilis* increased growth and reduced the concentration of *Cucumber mosaic virus* (Maldonado *et al.*, 2008).

Ramírez *et al.* (2006) observed in tomato plants 50 days after inoculation that the concentration of tomato spotted wilt virus (TSWV) decreased with the sprinkling of ASA, honey, molasses or the combination of ASA + molasses and ASA + honey. Mejía *et al.* (2009) found that when the ASM resistance inducer (Boost®) was applied before virus inoculation, it reduced the incidence of virosis in tree tomato by 50%.

The effect of *Bacillus* spp. in reducing the expression of symptoms caused by diseases of viral origin has been reported, in addition to an increase in height and fresh weight of plants, it can be attributed to induced systemic resistance (Samaniego, 2017). In relation to the above, it is important to evaluate management strategies aimed at the application of resistance inducers because the information on resistance/tolerance to diseases caused by viruses is scarce.

The objective of the research was to evaluate the effect of resistance inducers on viral concentration (optical density), root length, height, severity, total dry weight (leaf + stem + root), number and weight of fruits in plants infected with TMV in tomato cultivation (*Solanum lycopersicum* L.)

## Materials and methods

The development of the crop was under tunnel in the municipality of Tlapa de Comonfort, Guerrero state, from January to April 2018. To prepare the substrate, a homogeneous mixture of 4 packages of perlite plus 1 package of moss pedestrians was made (4:1 v/v) and the 6" terracotta plastic pots were filled with the substrate. Tomato seedlings were transplanted on January 16, 2018. Before transplantation, Confol (imidacloprid) foliage was sprayed at a dose of 1 mL 1 000<sup>-1</sup> seedlings, for pest insect control.

The inoculum was obtained from *Nicotiana occidentalis* infected by Tobacco mosaic virus (TMV) from the greenhouse of phytopathogenic viruses, Department of Agricultural Parasitology of the Autonomous University Chapingo, the pathogenicity of TMV was corroborated by inoculations in *Nicotiana glutinosa* plants (indicator plants) where was able to infect and develop the symptoms of local lesions (Holmes, 1929).

The inoculation of TMV was performed mechanically as follows: first 1 g of leaf tissue of the diseased plant with TMV showing symptoms, plus 10 mL of buffer solution, was first macerated in a plastic bag (11 × 17 cm) Phosphates 0.025M pH 7.2 + DIECA (sodium diethyldithiocarbamic acid), as a stabilizer, subsequently carborundum 600 meshes were applied on 3 tomato leaflets (one from the apical part and two from the middle part of the plant).

Then with a cotton extract, the virus was taken and applied to the previously sprinkled leaflets, by rubbing on the plants of all treatments except in the absolute control (healthy plant). Three days before the inoculation of the TMV resistance inducers were applied to the foliage (to have a plant with greater defense against viral infection) and after the inoculation they were sprayed every 12 days, for three times in total.

21 composite samples of the experiment were obtained at 38 days after inoculation (ddi), of each treatment and repetition consisted of taking a sample through the three plants of the experimental unit. It was performed by collecting leaflets of the apical shoots of each of the three plants where the symptoms were shown until a gram of plant tissue was obtained, placed in a 11 × 17 cm plastic bag, labeled by treatment number, repetition and date.

The serological analysis of the samples was carried out in the Agricultural Virology Laboratory of the Department of Agricultural Parasitology of the Chapingo Autonomous University. The DAS-ELISA test was performed, using antiserum, conjugate, positive and negative for TMV obtained from Agdia Inc. (Elkhart, Indiana) following the Clark and Adams protocol modified by Sutula *et al.* (1986). Optical density values were recorded at a wavelength of 405 nm with an ELISA plate reader (Dynatech, minireader II).

The results were interpreted according to the following criteria: the reaction was considered as positive (presence of phytopathogen) if the optical density reading was greater than or equal to three times the average of the negative control, and if the negative control presents values on average of optical density less than 0.03, only those samples with optical densities greater than 0.1 will be considered positive (SAGAR, 1997).

A completely randomized design with 7 treatments and three repetitions was used, within which two controls were evaluated, the negative control without inoculation and without application of resistance inductors, and a positive control with inoculation and without application of inductors (Table 1). In the statistical analysis, analysis of variance and multiple comparisons of means were performed using the significant Tukey honest difference with a 5% level of significance. The experimental unit was 3 tomato plants.

**Table 1. Tomato treatments.**

Treatment	AP	12*	24	36
1	M	M	MC	MC
2	VS	VS	F	F
3	A	BS	M	VB
4	VB	VB	VB	VB
5	KD	SM	A	VS
6			TN	
7			TP	

M= messenger gold®; VS= Virus Stop®; A= Actigard®; VB= Virablock®; KD= Kendal®; SM= Stymulus® Maxx; BS= *Bacillus subtilis*; MC= MC Cream®; F= Potassium Phosphite; TN= negative control; TP= positive control. AP= preventive application of inductors 3 days before TMV inoculation; \* = days after inoculation, application of resistance inductors.

## Results and discussion

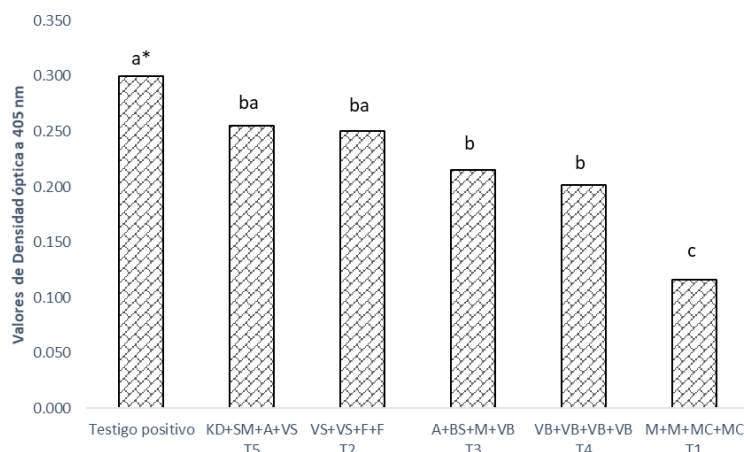
### Viral concentration values of optical density (OD)

In the analysis of variance significant differences were obtained between the treatments in the viral concentration of TMV, the Tukey means group showed for treatment 1 with the sequence of Messenger gold® (ia protein harpin aβ) + Messenger gold® + MC Cream® + MC Cream® the lowest

concentration of TMV (0.11 OD units) which was statistically different from the other treatments 5, 2, 3, 4 and positive control (Figure 1). Chuang *et al.*, 2014 mentioned the harpin protein in improving resistance to plant diseases.

The harpin protein are those fragments of pathogenic bacteria that plants recognize as bacteria, the harpin receptors can be found in all parts of the plant except in the woody bark, the harpin  $\alpha\beta$  protein quickly activates reactions in the plant after being recognized by the recipients of it. These receptors send hypersensitive responses (HR) or message; through the plant initiating a sequence of reactions such as induction of defense against various pathogens (Barón 2001; Fontanilla *et al.*, 2005; Choi *et al.*, 2013).

The resistance response could be due to the specific interaction of resistance genes (R) of the plant with the corresponding avirulence genes (Avr) of the pathogens (García and Lozoya, 2004). This defense reaction induced in the plant, can be to cope with conditions of biotic stress caused by TMV.



**Figure 1. Viral concentration (optical density) of TMV at 41 days after inoculation in tomato cv. Toro F1, treated with resistance inducers.** M= Messenger gold<sup>®</sup>, VS= Virus Stop<sup>®</sup>; A= Actigard<sup>®</sup>; VB= Virablock<sup>®</sup>; KD= Kendal<sup>®</sup>; SM= Stymulus<sup>®</sup> Maxx; F= potassium phosphite; BS= *Bacillus subtilis*; M = MC Cream. Source: own elaboration, 2019.

## Dry weight

For the variable dry leaf weight, significant differences were obtained, the highest weight (7.33 g) was recorded with the sprinkler of resistance inducers, T1 (Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup>); however, it was statistically not different from T2 (Virus Stop<sup>®</sup> + Virus Stop<sup>®</sup> + potassium phosphite + potassium phosphite) and statistically different from treatments 3, 4, 5 and positive control where the lowest weight occurred (Table 2).

There were no significant differences in stem weight; however, the greatest weight (3.64 g) was observed with the inducers (Virus Stop<sup>®</sup> + Virus Stop<sup>®</sup> + potassium phosphite + potassium phosphite) T2 (Table 2). In the variable root dry weight, a slight advantage was found with the spraying of (Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup>); however, it was statistically similar to the treatments (T2 and T3) and was statistically different from the positive control (Table 2).

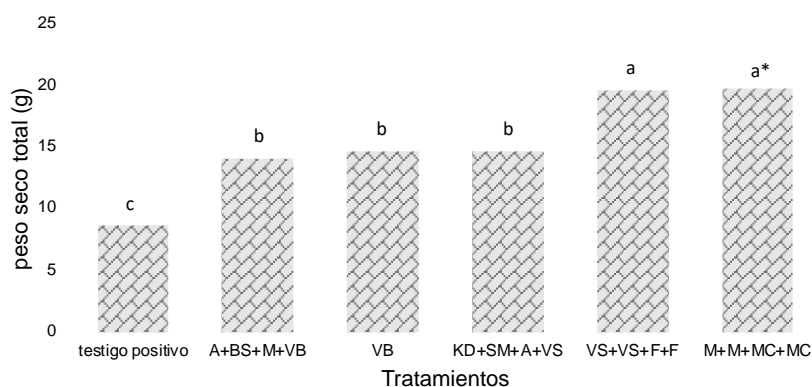
**Table 2. Dry weight of leaf, stem, root, total dry weight and root length, in tomato cv. Toro F1.**

Treatment	Leaf weight (g)	Stem weight (g)	Root weight (g)	Total dry weight (g)	Root length (cm)
T1	7.33 a*	3.46 a	8.95 a	19.74 a	43.74 a
T2	7.01 a	3.64 a	8.93 ba	19.59 a	39.97 ba
T3	4.51 b	3.02 a	6.52 bac	14.05 b	38.78 ba
T4	4.91 b	3.46 a	6.27 bc	14.64 b	38.78 ba
T5	5.29 b	3.25 a	6.14 c	14.68 b	29.67 bc
T7 (positive control)	2.86 c	2.39 a	3.41 d	8.66 c	23.63 c
DSH	1.42	1.42	2.65	4.53	10.66
Probability	0.0002	0.11	0.0001	<0.0001	0.0003

\* = means with the same letter by columns, are not statistically different according to the Tukey test at a  $p=0.05$ .

The variable total dry weight showed an increase in weight with resistance inductors T1 (Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup>) and T2 (Virus Stop<sup>®</sup> + Virus Stop<sup>®</sup> + potassium phosphite + phosphite of potassium), while the positive control recorded the lowest weight, 8.66 g. Research results mention elicitors or resistance inducers that promoted the accumulation of total biomass and increased height, in the tomato crop inoculated with *Fusarium oxysporum* f. sp. *lycopersici* (García *et al.*, 2018).

Maldonado *et al.* (2008) through resistance inductors reported an increase in size and weight of fresh biomass in squash plants inoculated with *Cucumber mosaic virus* that were treated with *B. subtilis* applied to the soil and acetylsalicylic acid applied to the foliage.



**Figure 2. Total dry weight (g) in tomato cv. Toro F1.** Resistance inductors are identified with the following keys: M= Messenger gold<sup>®</sup>, VS= Virus Stop<sup>®</sup>, A= Actigard<sup>®</sup>, VB= Virablock<sup>®</sup>, KD= Kendal<sup>®</sup>, SM= Stimulus<sup>®</sup> Maxx, F= potassium phosphite, BS= *Bacillus subtilis*, MC= MC Cream.

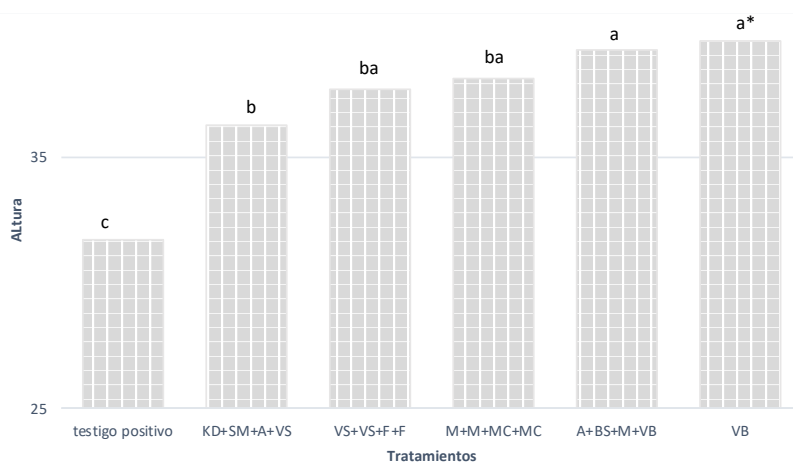
## Root length

Significant differences between treatments were obtained in the analysis of variance. The Messenger gold<sup>®</sup> resistance inducer in sequencing with MC Cream<sup>®</sup> (T1) obtained the greatest root length, compared to the positive control which showed the shortest length (Table 2). This could be

because Messenger gold<sup>®</sup> activates genes for plant growth and development (PHC, s/f). It has been reported that resistance inducers promote root growth, increased root growth was observed by imbibition of tomato seeds in chitosan solutions (González *et al.*, 2014).

## Height

Significant differences were found in the combined analysis through the three evaluations, the highest height 39.63 cm was found by T4 resistance inducers (Virablock<sup>®</sup> + Virablock<sup>®</sup> + Virablock<sup>®</sup> + Virablock<sup>®</sup>); however, it was statistically not different from treatments 3, 1 and 2, compared with the positive control, it increased 7.95 cm (Figure 3).

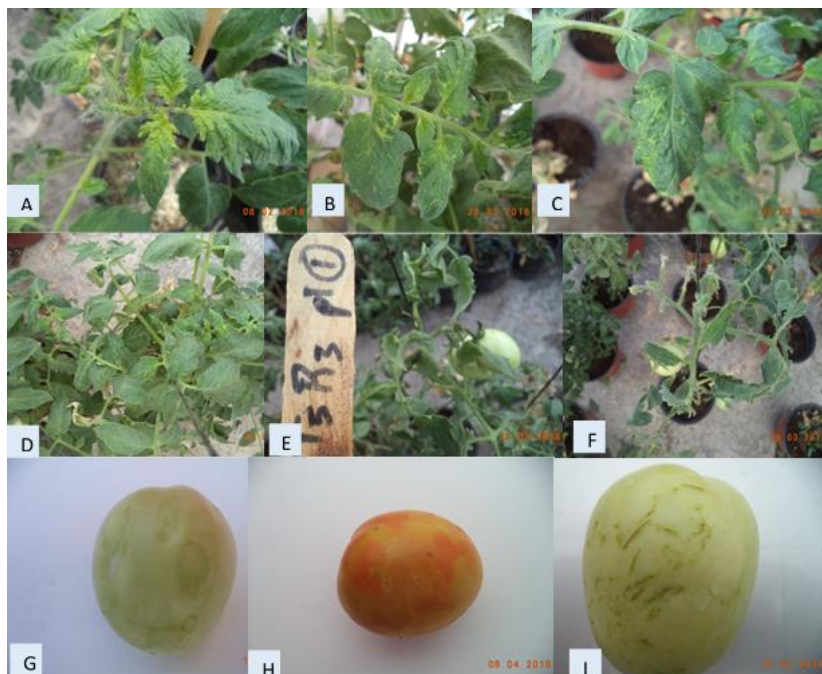


**Figure 3. Analysis of variance combined through the three dates of evaluation of the height in tomato.** M= Messenger gold<sup>®</sup>; VS= Virus Stop<sup>®</sup>; A= Actigard<sup>®</sup>; VB= Virablock<sup>®</sup>; KD= Kendal<sup>®</sup>; SM= Stymulus<sup>®</sup> Maxx; F= potassium phosphite; BS= *Bacillus subtilis*; MC= MC Cream.

Results of work with resistance inducers showed increases in height, Gonzalez *et al.* (2015) who observed a 14.3% increase with the application of *H. longipes* extract (inducing metabolites) in tomato plants inoculated with *Fusarium oxysporum*. García *et al.* (2018) found that applications of the elicitor of natural origin increased the height in tomato. Salamanca and Alvarado (2012) obtained the highest height with resistance inducer sprays (har protein).

Different symptoms were observed after the inoculation of TMV, an irregular yellow mosaic in the leaflets of the upper bud of the plant, then the mosaic was shown in the middle part of the leaf, the fruits presented irregular edges in the form of rings, yellow spots of intense color covering the fruit, deformed fruits, fall and wilting of flowers, dwarfism, leaf distortion and yield reduction (Figure 4).

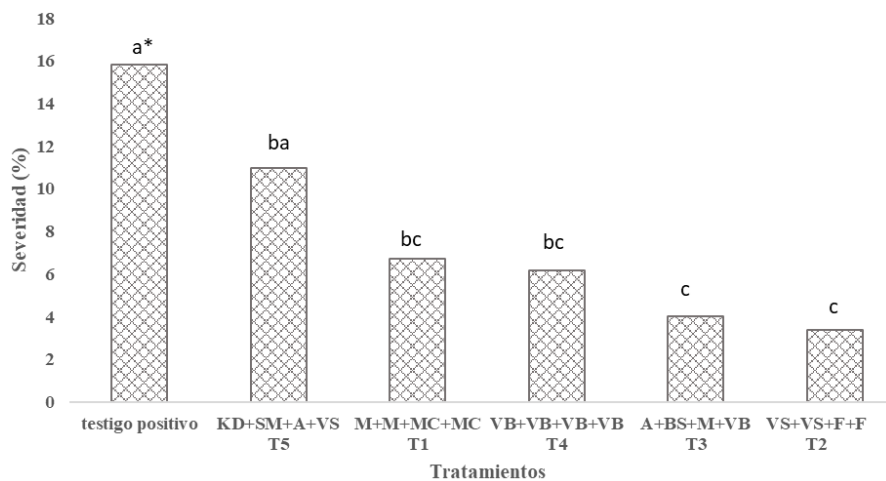
The observed symptomatology partially agrees with that mentioned by Velásquez *et al.* (2012) who found dwarfism, chlorosis, deformation of leaves (blistering, wavy or curly edges, lanceolate leaves, small in size), defoliation, leaf necrosis and branches attached to chili plants. Delgado (1974) includes mosaic, coriaceous consistency sheets, and abortion of buttons, symptoms similar to what was found in the present study.



**Figure 4.** A, B and C) folioles with chlorosis and mosaic; D) plant dwarfism; E) leaf distortion; F) abortion of flowers, G) fruits with rings; H) fruits with yellow spots; and I) deformed fruits.

### Severity

The results of the analysis of variance for severity showed significant differences between treatments. In the analysis of means, significant differences between resistance inductors were observed, the sequences of applications T2 (Virus Stop<sup>®</sup> + Virus Stop<sup>®</sup> + potassium phosphite + potassium phosphite) and T3 (Actigard<sup>®</sup> + *Bacillus subtilis* + Messenger gold<sup>®</sup> + Virablock<sup>®</sup>) were found recorded the lowest severity after three evaluations, compared to T5 and the positive control (Figure 5).



**Figure 5.** Analysis of variance combined through the three dates of evaluation of severity (%) in tomato.



It could probably be due to resistance inducers caused several defense reactions, such as the production of phytoalexins, antimicrobial proteins, pathogenicity-related proteins, oxidation reactions and structural changes at the cell wall level (Riveros, 2001; García and Lozoya, 2004).

### Number of fruits

Table 3 shows the results of the analysis of variance of the variable number of fruits, of the first, second and third cut according to the symptomatology observed in the fruits. In the first cut when applying inductors, Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup> (T1) obtained 1.67 fruits with yellow spots, statistically different from the positive control. Fruits were found that showed no symptoms with the inductors T1 (Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup>) and T4 (Virablock<sup>®</sup>) and statistically not unlike the negative control (T6) (Table 3).

**Table 3. Analysis of individual variance of the number of fruits according to the symptoms, first, second and third cut in tomato.**

Trat	First cut			Second cut				Third cut			
	FD	FCMA	FNPS	FD	FCA	FCMA	FNPS	FD	FCA	FCMA	FNPS
T1	0.33 a*	1.67 bc	2.33 ba	0 b	0.67 a	0 b	2 ba	1.33 a	0.33 a	2.33 ba	2.33ba
T2	0.33 a	3.67 ba	1 b	0 b	0.33 a	1.33 ba	1.33 ba	1.67 a	0.67 a	1.33 ba	3.33ba
T3	0 a	3.33 ba	1 b	0 b	0 a	0.33 ba	1.33 ba	0.67 a	0 a	2 ba	1.33 b
T4	0 a	2.33 b	2.33 ba	0 b	0 a	0.67 ba	0.33 ba	2.33 a	0 a	0.67 ba	1.67 b
T5	0 a	2.67 b	2 b	0 b	0.67 a	0.67 ba	0.67 ba	2.67 a	0.67 a	2.66 a	0.67 b
T6	0 a	0 c	5 a	0 b	0 a	0 b	3.33 a	0 a	0 a	0 b	7.67 a
T7	0 a	5 a	0 b	1 a	1 a	3.33 a	0 b	2.33 a	1 a	3.67 a	0 b
DSH	0.86	2.02	2.72	0	1.05	3.276	3.1	3.33	1.49	3.276	5.771
Prob.	0.56	<0.0001	0.0007	<0.0001	0.027	0.0445	0.0384	0.1328	0.1883	0.0287	0.009

FD = deformed fruits; FCMA= fruits with yellow spots, FNPS= fruits that showed no symptoms; FCA= fruits with rings; \* = means with the same letter are not statistically different according to the Tukey test at  $p=0.05$ .

In the second cut the application of inductors did not register deformed fruits compared to the positive control. The variable number of fruits with rings in the comparison of means does not show statistical differences. The sequence of inductors (Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup>) (T1) did not register fruits with yellow spots and was statistically different from the positive control. The use of resistance inducers showed statistically similar results to the healthy plant (negative control) in the number of fruits without symptoms (Table 3).

In the third cut the use of resistance inducers did not influence the number of deformed fruits, fruits with rings, fruits with yellow spots and fruits that showed no symptoms. However, in the variable fruits that did not show symptoms, a slight advantage is shown numerically through the use of sequence resistance inducers (Virus Stop<sup>®</sup> + Virus Stop<sup>®</sup> + potassium phosphite + potassium phosphite) (T2) obtained 3.33 fruits, statistically not different from the negative control (healthy plant) (T6) (Table 3).

In the analysis of combined variance, significant differences were found between the treatments in the variables number of fruits with yellow spots, number of fruits that did not show symptoms and number of fruits with rings (Table 4). In the variable deformed fruits, the applications of resistance inductors (Actigard<sup>®</sup> + *Bacillus subtilis* + Messenger gold<sup>®</sup> + Virablock<sup>®</sup>) of T3 showed a numerical advantage with the lower average of deformed fruits (0.22).

**Table 4. Analysis of combined variance of the number and weight of fruits according to the symptoms in tomato.**

Trat	Number				Weight			
	FD	FCMA	FNPS	FCA	FD	FCMA	FNPS	FCA
T1	0.55 a*	1.33 cb	2.22 b	0.5 ba	8.67 a*	43.22 bc	73.78 b	15 ba
T2	0.67 a	2.11 b	1.89 b	0.5 ba	17.22 a	58.67 ba	39.67 cb	16.34 ba
T3	0.22 a	1.89 b	1.22 b	0 b	3.78 a	57.89 ba	27.11 cb	0 b
T4	0.78 a	1.22 cb	1.44 b	0 b	12.22 a	35.22 bc	36.55 cb	0 b
T5	0.89 a	2 b	1.11 b	0.67 ba	13.11 a	53.56 ba	28.11 cb	20 ba
T6	0 a	0 c	5.33 a	0 b	0 a	0 c	153.13 a	0 b
T7	1.11 a	4 a	0 b	1 a	19.67 a	97.56 a	0 c	32 a
DSH	1.14	1.61	2.29	0.82	19.75	45.07	52.4	25.75
Prob.	0.065	<0.0001	<0.0001	0.0022	0.045	<0.0001	<0.0001	0.0019

FD= deformed fruits; FCMA= fruits with yellow spots; FNPS= fruits without symptoms; FCA= fruits with rings; \*= means with the same letter by columns, are not statistically different according to the Tukey test at a  $p= 0.05$ .

The lowest number of fruits with yellow spots (1.22) was found with the application of Virablock<sup>®</sup> (T4), statistically different from the positive control. 2.22 fruits were obtained that showed no symptoms by sprinkling inductors (Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup>) (T1), statistically not unlike the other inductors (T2, T3, T4, and T5). The treatments T3 (Actigard<sup>®</sup> + *Bacillus subtilis* + Messenger gold<sup>®</sup> + Virablock<sup>®</sup>) and T4 (Virablock<sup>®</sup>) showed 0 fruits with rings, statistically different from the positive control.

The results found suggest that applications of resistance inducers in tomato cultivation reduce the number of fruits with expression of TMV symptoms. As indicated by Baysal *et al.* (2003) one of the potential methods of reducing the severity of diseases caused by pathogens is the induction of resistance in the plant and González *et al.* (2015) report 80% decrease in severity of *Fusarium oxysporum* f. sp. *Lycopersici* on symptoms of wilting in tomatoes through the use of inducing metabolites (*Heliopsis longipes* extract).

### Fruit weight

The analyzes of variance of the first, second and third cut of the weight of fruits were obtained based on the symptoms that the fruits presented (Table 5).

**Table 5. Analysis of individual variance of the weight (g) of fruits according to the symptoms, first, second and third cut in tomato.**

Trat	First cut			Second cut				Third cut			
	FD	FCMA	FNPS	FD	FCA	FCMA	FNPS	FD	FCA	FCMA	FNPS
T1	8.33 a*	66.33ba	95.33 b	0 b	23.33a	0 b	71 ba	17.67a	6.67 a	63.33ba	55 b
T2	12.67a	115 a	27 cb	0 b	10 a	32.33ba	27 ba	39 a	22.67a	28.67ba	65 ba
T3	0 a	117.67a	16 c	0 b	0 a	6.33 b	39 ba	11.33a	0 a	49.67ba	26.33 b
T4	0 a	82.67 a	60 cb	0 b	0 a	9.67 ba	9.33 b	36.67a	0 a	13.33ba	40.33 b
T5	0 a	85 a	52.33cb	0 b	25 a	10 ba	20 ba	39.33a	15 a	65.67ba	12 b
T6	0 a	0 b	179.39a	0 b	0 a	0 b	106.67a	0 a	0 a	0 b	173.33a
T7	0 a	124.33a	0 c	15.67 a	36.67a	61.67 a	0 b	43.33a	27.33a	106.67a	0 b
DSH	27.67	73.479	76.47	4.26	37.13	52.69	91.214	54.119	40.62	93.49	109.69
Prob.	0.55	0.0007	<0.0001	<0.0001	0.02	0.012	0.016	0.0958	0.1556	0.0252	0.002

FD= deformed fruits; FCMA= fruits with yellow spots; FNPS= fruits without symptoms; FCA= fruits with rings; \* = means with the same letter by columns, are not statistically different according to the Tukey test at a  $p= 0.05$ .

The analysis of variance of the first cut showed significant differences between treatments in two variables weight of fruits with yellow spot and weight of fruits that showed no symptoms. The resistance inducers did not influence deformed fruit weight and weight of fruits with yellow spots, however numerically in the variable weight of fruits with yellow spots, the lowest weight 66.33 was found with the sequence of inductors (Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup>).

In comparison to the positive control treatment where the highest weight 124.33 g occurred and in the variable fruits that showed no symptoms, it showed the inducers of treatment 1 (Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup>) which registered 95.33 g, statistically different from the positive control (Table 5). In the second cut the resistance inducers did not influence for the variables weight of deformed fruits, weight of fruits with rings, weight of fruits with yellow spots and weight of fruits that showed no symptoms.

However, for the variable fruit with rings, 0 g was observed with the sprinkling of resistance inducers of T3 (Actigard<sup>®</sup> + *Bacillus subtilis* + Messenger gold<sup>®</sup> + Virablock<sup>®</sup>) and T4 (Virablock<sup>®</sup>). In the weight of fruits with yellow spots, 0 fruits were obtained in T1 (Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup>) and was statistically different from the positive control.

The variable weight of fruits that showed no symptoms shows that applications of resistance inducers of T1 (Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup>) obtained the second highest weight 71 g. In relation to deformed fruits, inducer applications showed 0 g (Table 5). In the third cut, differences were obtained numerically with the applications of resistance inducers, for the variables weight of deformed fruits, weight of fruits with rings, weight of fruits with yellow spots and weight of fruits that showed no symptoms.

It found in the analysis of combined variance significant differences between treatments in the variables weight of deformed fruits, weight of fruits with yellow spots, weight of fruits that showed no symptoms, and weight of fruits with rings; however, the sprinkling of resistance inductors showed differences numerically (Table 4). The lowest weight of deformed fruits 3.78 g was recorded in the sequence (T3) (Actigard<sup>®</sup>+*Bacillus subtilis*+Messenger gold<sup>®</sup>+Virablock<sup>®</sup>).

The inductor Virablock<sup>®</sup> (T4) obtained the lowest weight of fruits with yellow spots registered 35.22 g, in the variable weight of fruits that did not show symptoms, the inductors of resistance Messenger gold<sup>®</sup>+Messenger gold<sup>®</sup>+MC Cream<sup>®</sup>+MC Cream<sup>®</sup> (T1) obtained the second highest weight 73.78 g; In relation to the variable weight of fruits with rings, 0 g were obtained using the inductors of T3 (Actigard<sup>®</sup>+ *Bacillus subtilis*+Messenger gold<sup>®</sup>+Virablock<sup>®</sup>) and T4 (Virablock<sup>®</sup>) (Table 4).

The results obtained through the application of resistance inductors show lower number and weight in fruits compared to the control inoculated with TMV and without inductor applications. Favorable results have been found in the management strategy through the application of resistance inductors in tomato plants as mentioned by Pérez *et al.* (2017) who treated with Acibenzolar S-methyl (ASM) and acetylsalicylic acid (ASA), which presented the lowest levels of incidence and severity of virosis at the different evaluation dates under greenhouse conditions.

## Conclusions

Inductors (Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup>) of treatment one, at 38 days after inoculation reduced the concentration of Tobacco mosaic virus based on ELISA results. Virablock<sup>®</sup> spray after the three evaluations obtained a higher average height. The resistance inductors (Virus Stop<sup>®</sup> + Virus Stop<sup>®</sup> + potassium phosphite + potassium phosphite) obtained the lowest average severity.

The highest total dry weight was obtained with the inductors (Messenger gold<sup>®</sup> + Messenger gold<sup>®</sup> + MC Cream<sup>®</sup> + MC Cream<sup>®</sup>) and the greatest root length at the end of the crop; presented the second highest number and average weight of fruits that showed no symptoms of *Tobacco mosaic virus*, after the three cuts.

## Cited literature

- Barón, J. 2001. Estrategias de manejo de la resistencia. IR-4 Project. Eden Bioscience, WA, USA. 35 p.
- Bautista, N.; Chavarrín, C. y Valenzuela, F. 2010. Jitomate: tecnología para su producción en invernadero. Colegio de Postgraduados. Montecillos, México. 49-63 pp.
- Baysal, Ö.; Soyly, E. M. and Soyly, S. 2003. Induction of defence-related enzymes and resistance by the plant activator acibenzolar-S-methyl in tomato seedlings against bacterial canker caused by *Clavibacter michiganensis* ssp. *michiganensis*. Reino Unido. Plant Pathol. 52(6):747-753.
- Choi, M. S.; Kim, W.; Lee, C. and Oh, C. S. 2013. Harpins, multifunctional proteins secreted by gram-negative plant-pathogenic bacteria. Molecular plant-microbe interactions. 26(10):1115-1122.

- Chuang, H.; Chang, P. y Syu, Y. 2014. Harpin protein, an elicitor of disease resistance, acts as a growth promoter in *Phalaenopsis* orchids. *J. Plant Growth Regul.* 33(4):788-797. Doi: 10.1007 / s00344-014-9425-1.
- Delgado, S. S. 1974. Los virus que atacan el cultivo del chile en México; sus implicaciones, identificación, transmisión y medidas de combate. *Agríc. Téc. Méx.* 3(1):317-325.
- Ebel and Cosio, E. G. 1994. 'Elicitors of plant defense responses' *International Review of Cytology.* 148(1):1-36.
- FAO-FAOSTAT. 2018. Food and Agriculture Organization of the United Nations. Trade. Recuperado de la red: <http://faostat.fao.org/site/342/default.aspx>.
- FIRA. 2019. Panorama Agroalimentario. Dirección de Investigación y Evaluación Económica y Sectorial. Tomate rojo. México. 25 p.
- Fontanilla, M.; Montes, M. and De Prado. R. 2005. Effects of the foliar-applied protein "Harpin (Ea)" (messenger) on tomatoes infected with *Phytophthora infestans*. *Communications in Agric. Appl. Bio. Sci.* 70(3):41-45.
- García, E. E. L.; Robledo, O. A.; Benavides, M. A.; Solís, G. S. y González, M. S. 2018. Efecto de elicitores de origen natural sobre plantas de tomate sometidas a estrés biótico. *Rev. Mex. Cienc. Agríc. Pub. Esp.* 20:4211-4221.
- García, P. E. y Lozoya, G. E. 2004. Genes de resistencia a enfermedades en plantas. *Rev. Mex. Fitopatol.* 3(22):414-422.
- González, M. S.; Benavides, M. A.; García, E. E. L.; Rodríguez, C. E. M. y Flores, O. A. 2015. Efecto de las alcalmidas como inductores de tolerancia al estrés biótico en tomate. *Rev. Mex. Cienc. Agríc. Pub. Esp.* 12:2371-2382.
- González, P. D.; Costales, D.; y Falcón, A. B. 2014. Influencia de un polímero de quitosana en el crecimiento y la actividad de enzimas defensivas en tomate (*Solanum lycopersicum* L.). *Cultivos Tropicales.* 35(1):35-42.
- Hanssen, I. M.; Lapidot, M. and Thomma, B. P. 2010. Emerging viral diseases of tomato crops. *Mol Plant Microbe Interact.* 23(5):539-548.
- Holmes, F. O. 1929. Local Lesions in *Tobacco Mosaic*. *Botanical Gazette.* 87(1):39-55.
- Ley, F. J. H. y García, E. R. S. 1998. Virus que afectan al cultivo de jitomate. *In: enfermedades de las hortalizas*, Cruz, O. J. E.; García, E. R. S. y Carrillo, F. J.A. (Eds.). Universidad Autónoma de Sinaloa, Sinaloa, México. 79-83 pp.
- Maldonado, C. E.; Ochoa, M. D. L. y Tlapal, B. B. 2008. Efecto del ácido acetil salicílico y *Bacillus subtilis* en la infección causada por *Cucumber mosaic virus* en calabacita. *Rev. Chapingo Ser. Hortic.* 14(1):55-59.
- Mejía, D. M.; Rodas, E. I.; Patiño, L. F. y González, E. P. 2009. Efecto del acibenzolar-s-metil sobre el desarrollo de la virosis causada por potyvirus en tomate de árbol. *Agron. Colomb.* 27(1):87-93.
- Pérez, R. M.; García, E. P.; Durán, T. Y.; Luna, C. A.; Loera, A. E. y Pérez, R. A. 2017. Control alternativo de virosis en jitomate en un invernadero comercial de Zacatepec, Morelos. *Rev. Mex. Fitosanidad.* 1(1):1-12.
- PHC. s/f. Plant Health Care De México. PHC® Messenger Gold®. <http://www.phcmexico.com.mx/pdfs/biopesticidas/Messenger%20Gold.pdf>.
- Pieterse C., Van Wees S. 2015. Resistencia a enfermedades inducidas. *In: Lugtenberg B. (Eds.). Principios de interacciones planta-microbio.* Springer Cham. 123-133 pp. DOI: [https://doi.org/10.1007/978-3-319-08575-3\\_14](https://doi.org/10.1007/978-3-319-08575-3_14).

- Ramírez, F. J.; Ochoa, M. D. L.; Rodríguez, M. M. N. y Mora, A. G. 2006. Efecto del ácido acético salicílico, miel y melaza en la movilidad y concentración de TSWV. *Rev. Chapingo Ser. Hortic.* 12(2):239-243.
- Riveros, A. A. S. 2001. Moléculas activadoras de la inducción de resistencia, incorporadas en programas de agricultura sostenible. *Manejo Integrado de Plagas. Costa Rica.* 61:4-10.
- SAGAR. 1997. Subsecretaría de Agricultura y Ganadería. Guía ilustrada de la prueba de inmunoabsorción con enzimas ligadas para la detección de fitopatógenos. México. 22 p.
- Salamanca, C. M. and Alvarado, G. A. 2012. Effect of harpin protein and potassium phosphite in control powdery mildew (*Frysiphe polygoni* D. C.) in tomato of Sutamarchán (Boyacá). *Ciencia y Agricultura.* 9(2):65-75.
- Samaniego, G. B.; Reyes, R. A.; Moreno, V. O. A. y Tun, S. J. M. 2017. Resistencia sistémica inducida contra virus fitopatógenos mediada por la inoculación con la rizobacteria *Bacillus* spp. *Rev. Protección Veg.* 32(1):10-22.
- Schreiber, K., and Desveaux, D. 2008. Message in a bottle: chemical biology of induced disease resistance in plants. *Plant Pathol. J.* 24(3): 245-268.
- SIAP. 2018. Anuario estadístico de la producción agrícola 2003-2016. Recuperado de la red: <http://nube.siap.gob.mx/cierre-agricola/>.
- Sikora, E. J. 2011. Virus diseases of tomato. Alabama Cooperative Extension System. ANR-0836. Department of Plant Pathology. Alabama A & M and Auburn Universities. 6 p. <https://docplayer.net/22918775-Virus-diseases-affect-tomato-production-to-some.html>.
- Sutula, CH. L.; Gillett, J. M.; Morrissey, S. M. y Ramsdell, D. C. 1986. Interpreting ELISA Data and Establishing the Positive - Negative Threshold. *Plant Dis.* 70(8):722-726.
- Thakur y Singh, 2012. Role of elicitors in inducing resistance in plants against pathogen infection: a review. *ISRN Biochemistry.* ID 762412, 10 p.
- Vallad, G. E. and Goodman, R. M. 2004. Review & interpretation in conventional agriculture. *Crop Sci.* 44(6):1920-1934.
- Velásquez, V. R.; Reveles, T. L. R. y Mena, C. J. 2012. Incidencia y sintomatología de cinco virus en parcelas comerciales de chile seco en Aguascalientes, San Luis Potosí y Zacatecas, México. *Rev. Mex. Cienc. Agríc.* 2(3):381-390.