Rev. FCA UNCUYO. 2019. 51(1): 161-171. ISSN (en línea) 1853-8665.

Salicylic acid and *Bacillus subtilis* as control of early blight (*Alternaria solani*) in tomato plants (*Solanum lycopersicum*)

Ácido salicílico y *Bacillus subtilis* como control del tizón temprano (*Alternaria solani)* en plantas de tomate (*Solanum lycopersicum*)

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Originales: *Recepción:* 29/11/2017 - *Aceptación:* 11/10/2018

Abstract

Alternaria solani is the causal agent of early blight disease in tomatoes (*Solanum lycopersicum*) and every year is responsible for significant economic losses suffered worldwide by the producers of this crop. Since salicylic acid and *Bacillus subtilis* are resistance-inducing agents in plants, they were evaluated in order to know their effect on the infection caused by early blight in the tomato crop. Plants of 75 days old were transplanted in furrows 8 m wide by 43.2 m long, the exogenous application of treatments were made, growth variables (plant height and stem diameter), yield (total fruits) and damage in tomato plants were evaluated. The results show significant statistical differences between treatments compared with the control ($P \leq 0.000$). *B. subtilis* applied to the root and salicylic acid applied to the foliage, reduced the severity of *A. solani* in tomato plants, and caused a significant increment in the crop growth and yield. The use of resistance inducers can represent an alternative of sustainable production and efficient control against pathogens, aimed at reducing the use of agrochemicals and production costs.

Keywords
inductors of resistance • *Alternaria solani* • sustainable production

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Resumen

Alternaria solani es el agente causal de la enfermedad del tizón temprano en el tomate (*Solanum lycopersicum*) y cada año es responsable de pérdidas económicas significativas sufridas por los productores de este cultivo a nivel mundial. Debido a que el ácido salicílico y *Bacillus subtilis*, son agentes inductores de resistencia en las plantas, estos fueron evaluados con la finalidad de conocer su efecto en la infección causada por el tizón temprano en el cultivo de tomate. Plantas de 75 días de edad se trasplantaron en surcos de 8 m de ancho por 43,2 m de largo, se realizó la aplicación exógena de los tratamientos, se evaluaron variables de crecimiento (altura de la planta y diámetro del tallo), rendimiento (frutos totales) y daño en plantas de tomate. Los resultados muestran que existen diferencias estadísticas significativas entre los tratamientos comparados con el testigo ($P \leq 0{,}000$). *B. subtilis* aplicado a la raíz y ácido salicílico aplicado al follaje, redujo la severidad de *A. solani* en plantas de tomate, al mismo tiempo que causó un incremento significativo en el crecimiento y rendimiento del cultivo. La utilización de inductores de resistencia puede representar una alternativa de producción sustentable y eficiente para el control de patógenos, a fin de reducir el uso de agroquímicos y los costos de producción.

Palabras clave

inductores de resistencia • *Alternaria solani* • producción sustentable

Introduction

In Mexico, the tomato (*Solanum lycopersicum* L.*)* is one of the most important edible and nutritious plant crops and sources of income (29). Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food reported that the tomato is the agricultural product of major exportation in the country, between January and October 2016 it got an income of 1 742 million dollars, which represented a growth at an annual rate of 15 percent (40).

The expected production of tomato in the State of Chiapas was of 47,736 t (42), by the spring-summer period of 2017. Specifically, in the plateau of Comitán, the production of tomato is 31,224.90 t/year (10,320 t/year in La Trinitaria) (41).

The vulnerability of growers engaged in the production of tomatoes that face health phenomena (pests or diseases) is a reality since they depend on the primary sector. The pests' invasion has been a disturbing phenomenon in nature, whose ecological and economic consequences are the negative effect on the ecosystem (1, 4).

In agriculture, fungal diseases have a devastating effect on food production. Among the diseases that affect the tomato crop, it can find the anthracnose caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., late blight caused by *Phytophthora infestans* (Mont.) of Bary and early blight caused by *Alternaria tomatophila* E. G. Simmons and *Alternaria solani* Sorauer (7, 37).

Early blight, caused by the fungus *Alternaria solani* Sorauer, is one of the most important tomato diseases worldwide (33). It can affect foliage, stems, and fruits of the infected plants (16). Early blight is responsible for a large proportion of the total monetary losses suffered by tomato producers each growing season. If the incidence of the disease is high, the fungus can cause extensive defoliation, which leads to a reduction in the economic performance of the crop (16). Historically, the control of early blight disease has been achieved mainly through the application of chemical fungicides, long crop rotations and efforts to cultivate resistant tomato varieties (3, 12, 16, 27).

Salicylic acid can be particularly valuable for crops of hydroponic tomatoes susceptible to infection by *A. solani* and *Botrytis cinerea* (gray mold) (17), since its exogenous application activates the acquired systemic resistance against *A. Solani* (44). Tobacco plants resistant to the Tobacco mosaic virus synthesize various pathogenesis related proteins soon after infection and exogenous application of salicylic acid induces the expression of the genes and the resistance of plants to virus (21) or other phytopathogens such as bacteria and fungi (5).

In pumpkins, it has also been found that salicylic acid could function as a compound that transmits the resistance signal against Tobacco necrosis virus (21, 24).

In addition to this, nowadays it is known that several species of bacteria of the genera Pseudomonas and Bacillus have been used to induce systemic resistance against diseases caused by different fungi, bacteria, nematodes and viruses in crops such as tomato, cucumber, chili, peanut and strawberry (9, 10, 11, 18, 39, 46, 47).

Objective

To evaluate the effect of *B. subtilis* and acetylsalicylic acid against early blight (*A. solani*) in tomato plants (*S. lycopersicum*), as an alternative to the application of fungicides in order to reduce the negative effect that these have on the ecosystem and on human health (36, 43, 45).

Materials and methods

This work was carried out in the plateau of Comitan, in the community of Victorico R. Grajales, Road to Lagos de Montebello at Km 26.5, municipality of La Trinitaria, Chiapas, Mexico, at 16°7'16.71" N and 91°49'0.48" W, an altitude of 1534 m a. s. l. (14).

Tomato transplant

Tomato plants of 75 days old, from the determined growth hybrid Pony Express F1 (Harris Moran®), were transplanted in 36 rows of 8 m wide by 43.2 m long (345.2 m^2), at a distance of 0.40 m between plants and 1.20 m among rows, with 20 plants per row and under a canopy of antiaphids mesh (15).

Treatments application

Nine treatments were established under a completely randomized design with four repetitions (each repetition with 20 tomato plants).

The treatments evaluated were: a) *B. subtilis* applied to the root; b) *B. subtilis* applied to the foliage; c) salicylic acid applied to the foliage; d) salicylic acid applied to the root; e) *B. subtilis* and salicylic acid applied to the foliage; f) *B. subtilis* and salicylic acid applied to the root; g) *B. subtilis* applied to the root and salicylic acid applied to the foliage;

h) Chemical control applied to the root and foliage, and i) control without application.

In the case of *B. subtilis*, the commercial product Probacil® was used, which consisted of a liquid concentrate of this bacterium with 100 million cells per mL of the LPbs1 strain.

The application was repeated weekly, by spraying at a dose of 10 mL / L of water with a manual sprinkler, starting from the transplant until the end of the experiment, 90 days after the transplant. For salicylic acid evaluation, the commercial product Aspirin Bayer® (500 mg) was used, at a dose of 1 g/L of water. The application of this product was carried out in the same way as *B. subtilis*, at a dose of 1 mL / L.

The chemical treatment was applied according to the different fungicides and doses usually used by the tomato producers of La Trinitaria (Plateau of Comitan), Chiapas: metalaxyl 25% EC (1 mL / L), gentamicin 2% + Oxytetracycline 6% WP (2 g / L), mancozeb 80% WP (10 g / L), chlorothalonil 72% + cymoxanil 8% WP (10 g / L), dimetomorph 25% + chlorothalonil 50% WG (8 mL / L) and tebuconazole 50% + trifloxystrobin 25% WG (1.25 mL / L). For the application of all the treatments tulle blankets were used between the plots, to avoid errors in the spraying of the treatments. The control consisted of tomato plants without application of the aforementioned products.

Evaluated variables

Plant growth, yield and disease damage were evaluated, during a period of 90 days after the transplant (30). Growth variables evaluated were plant height (cm) from ground level to the top of the plant, with the help of a Trupper® measuring tape; and stem thickness (cm) 5 cm above the substrate, for which a Santaley® digital vernier caliper was used.

The yield variable was total fruit production, by counting all the bunches for each plant. Finally, disease damage was evaluated, using the scale developed by the British Mycological Society, cited by Mendoza and Tórrez (2005) (table 1).

Statistical analysis

The normality test was carried out using the Shapiro Wilks test (34). Because of data abnormality, treatments comparison and the determination of the possible statistical differences between them were carried out by means of a Kruskal Wallis and Mann Whitney analysis (26). All calculations were made in the statistical package R and RStudio (32, 35).

Table 1. Damage scale based on British Mycological Society applied to tomato plants.

Tabla 1. Escala de daño basada en British Mycological Society, aplicada para las plantas de tomate.

Scale	%Damage	Foliar area with spots
0	0-0	the entire leaf area of the plant without spots
1	0.1-5	from 5 to 10 spots on the leaf area
2	6-10	¼ of the plant is affected
3	11-25	⅓ of the plant is affected
4	26-50	½ of the plant is affected
5	51-75	75% of the whole plant is affected
6	76-100	all dead leaves, dead or dying stems

Results and discussion

According to the studies, there were significant differences between the control and all the treatments for tomato plants height, except for salicylic acid treatment applied in the foliage ($K = 97.3$, $df = 8$, $p < 0.000$). Chemical treatment, *B. subtilis* applied to the root and salicylic acid applied to the foliage differed significantly from control for stem thickness ($K = 34.6$, $df = 8$, $p = 0.000$). Tomato plants showed significant differences between the control and all treatments in the number of fruits and disease damage ($K = 162.6$, $df = 8$, $p < 0.000$, $K = 96.5$, $df = 8$, $p < 0.000$, respectively) (figure 1). For plant height chemical fungicides was the most efficient treatment (74.2 cm ± 5.5), followed by *B. subtilis* applied to the root and salicylic acid applied to the foliage (70.1 cm ± 7.1), compared with the control (56.5 cm ± 5.2). Regarding the stem thickness, *B. subtilis* applied to the root (1.2 cm ± 0.1) presented the best results compared with the control (0.8 cm ± 0.1).

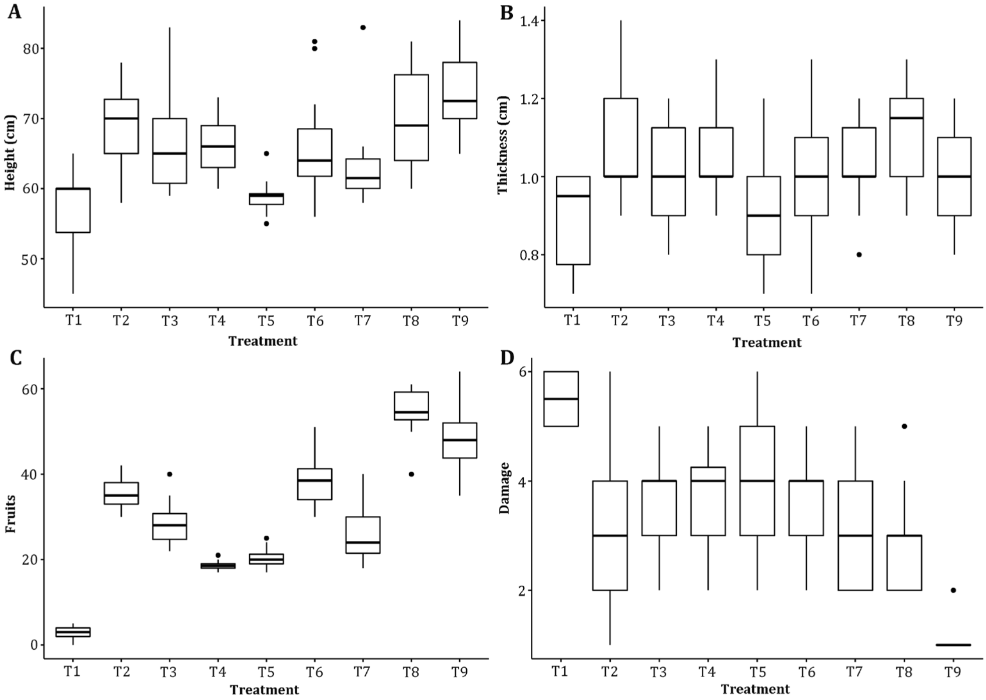

Figure 1. The difference in the distribution of the data of each treatment for the variables: Plant height (A), Stem thickness (B), Fruit number (C) and damage level (D).

Figura 1. Diferencia en la distribución de los datos de cada tratamiento para las variables: Altura de planta (A), Grosor del tallo (B), Número de frutos (C) y nivel de daño (D).

Tomato plants treated with *B. subtilis* applied to the root and salicylic acid applied to the foliage had a higher yield (55.0 ± 4.9) (photo 1, page 167).

Similar results have been reported by Avilés (2008), who achieved an increase in the tomato yield (1.38 kg/plant) with the application of salicylic acid at a concentration of 0.01 mM, compared to the control. Analyzing the performance of the lettuce crop (*Lactuca sativa* L.), it was observed that the treatment with salicylic acid (0.01 mM) caused an increase of 1.02 $kg.m^2$ with respect to the control (8).

In jalapeño pepper, salicylic acid in concentrations of 0.1 mM and 0.2 mM, showed an increase of root biomass (43%) and foliar biomass (36%); and an increase of 17% and 28% in fruit production in relation to the control (39). Matos (2004), evaluated applications of salicylic acid at concentrations of 0.005 and 0.01 mM in tomato plants and achieved an increase in the dry biomass of leaves, stem, and root. So also Mex *et al.* (2010) when using salicylic acid in concentrations of 1 μM in petunia increased the number of flowers by 72% and induced early flowering in comparison with the control. Applications of salicylic acid in the tomato crop significantly increased plant height, leaf area, stem fresh and dry weight, as well as the root length, perimeter and area (19). Crops yield is the main objective in any production (6). Vallad and Goodman (2004), mention that some inducers of resistance in addition to activating defense mechanisms promote a favorable effect on quality and yield on the fruits.

Regarding the damage caused by early blight, the treatment with chemical fungicides used by the producers was the most efficient (1.2 ± 0.4), however, the application of *B. subtilis* applied to the root and salicylic acid to the foliage reduced the damage caused by this fungus (2.8 ± 0.7), compared to the control (5.5 ± 0.5). Due to heavy rains and excess moisture in the study area, the fungus *Alternaria solani*, which causes an early blight on tomato plants, was observed 45 days after transplant (photo 2, page 167). It is worth mentioning that for all the treatments and variables evaluated, the results were outstanding in relation to the control (table 2, page 168).

Vallad and Goodman (2004) mention that some plant growth promoting rhizobacteria (PGPR) activate the jasmonic acid and ethylene routes, activating defense responses of the plants. With the use of these two resistance inducers, the severity of the pathogens was inhibited as observed in this study. Agreeing with works carried out by Ji *et al*. (2006), where they observed that 14 days after having inoculated PGPR (P. *fluorescens* 89B-61 and *Bacillus pumilus* SE34) in the soil at the time of transplant and after inoculation of tomato plants leaves with *Pseudomonas syringae* pv. tomato, the plant generated a protection of 60% and a greater protection (82.2%) when making a combination with P. *fluorescens* 89B-61. Raupach and Kloepper (2000), applied *Bacillus subtilis* strain GB03, *B. pumilus* INR7 and *Curtobacterium flaccumfaciens*, to evaluate induction of resistance system against *P. syringae pv. lachrymans* in cucumber crop. They observed a significant growth promotion in plants inoculated with these bacteria compared to the untreated control.

In zucchini plants (*Cucurbita pepo* L. var. Zucchini gray), mechanically inoculated with *Cucumber mosaic virus* (cucumber mosaic virus), the application of *B. subtilis* Biologic® at a dose of 5 mL/L to the soil and acetyl salicylic acid (1 g/L) to the foliage, generated a significant increase (21.4 g and 21.3 g respectively) in the weight of fresh biomass compared with the control (14.3 g).

Photo 1. Fruit yield in tomato plants treated with *B. subtilis* and salicylic acid applied to the root/foliage, in the plateau of Comitan, La Trinitaria, Chiapas, Mexico.

Foto 1. Rendimiento de frutos en plantas de tomate tratadas con *B. subtilis* y ácido salicílico aplicado a la raíz/follaje, en zona de la Meseta Comiteca, La Trinitaria, Chiapas, México.

Photo 2. Early blight disease caused by *Alternaria solani* in leaf (A) and stem (B) of tomato plants 45 days after transplant.

Foto 2. Enfermedad del tizón temprano causado por *Alternaria solani* en hoja (A) y tallo (B) de plantas de tomate a 45 días después del trasplante.

Table 2. Means and standard deviations for height, thickness, number of fruits and damage in each treatment evaluated for the control of *Alternaria solani* in tomato plants.
Tabla 2. Medias y desviaciones estándar por tratamientos para la altura, el grosor, número de frutos y daño evaluados para el control de *Alternaria solani* en plantas de tomate.

Tratamiento	Variable							
	Height (cm)		Thickness (cm) Fruit number Damage					
	X sd	M	X sd	M	X sd	M	X sd	M
T1	56.5±5.2 [e]	60.0	0.8±0.1 [c]	0.9	3.0±1.2 [f]	3.0	5.5±0.5 [e]	5.5
T2	69.4±5.6 [ab]	70.0	1.2±0.1 [ab]	1.0	35.1±3.5 [c]	35.0	3.1±1.4 [bc]	3.0
T3	66.3±6.6 [bc]	65.0	1.0±0.1 [abc]	1.0	28.8±4.7 [d]	28.0	3.4±0.8 [bcd]	4.0
T4	66.1±3.9 [bc]	66.0	1.1±0.1 [ab]	1.0	18.6±1.1 [e]	18.5	3.8±0.9 [cd]	4.0
T5	58.8±2.2 [de]	59.0	0.9±0.1 [bc]	0.9	20.4±2.1 [e]	20.0	4.1±1.3 [d]	4.0
T6	65.6±6.5 [bc]	64.0	0.9±0.2 [abc]	1.0	38.2±5.7 [c]	38.5	3.7±0.9 [bcd]	4.0
T7	63.1±5.3 [cd]	61.5	1.0±0.1 [ab]	1.0	26.0±6.5 [d]	24.0	3.3±1.1 [bcd]	3.0
T8	70.1±7.1 [ab]	69.0	1.1±0.1 [a]	1.3	55.0±4.9 [a]	54.5	2.8±0.7 [b]	3.0
T9	74.2±5.5 [a]	72.5	0.9±0.1 [abc]	1.0	47.8±6.4 [b]	48.0	1.2±0.4 [a]	1.0

X = Medium; sd = Standard deviation; M = Median; Media with unequal letters are statistically different.

X = Media; sd = desviación estándar; M = mediana; Medias con letras desiguales son estadísticamente diferentes.

Control (T1), *B. subtilis* applied to the root (T2*), B. subtilis* applied to the foliage (T3), salicylic acid applied to the root (T4), salicylic acid applied to the foliage (T5), *B. subtilis* and acid salicylic applied to the root (T6), *B. subtilis* and salicylic acid applied to the foliage (T7), *B. subtilis* applied to the root and salicylic acid applied to the foliage (T8), chemist applied to the root and foliage (T9).

Control (T1), *B. subtilis* aplicado a la raíz (T2), *B. subtilis* aplicado al follaje (T3), ácido salicílico aplicado a la raíz (T4), ácido salicílico aplicado al follaje (T5), *B. subtilis* y ácido salicílico aplicado a la raíz (T6), *B. subtilis* y ácido salicílico aplicado al follaje (T7), *B. subtilis* aplicado a la raíz y ácido salicílico aplicado al follaje (T8), químico aplicado a la raíz y follaje (T9).

In addition the application of *B. subtilis* reduced the concentration of *Cucumber mosaic virus* in plants (21). The results of the damage caused by *Alternaria solani* coincide with those of Tlatilpa (2010), who observed that *Bacillus subtilis* applied to the root controled the severity of *Clavibacter michiganensis subsp. michiganensis* and did not affect the growth or yield of tomato plants. However, it would be more convenient to apply *B. subtilis* to the root, than to the foliage, since higher yield and stem thickness can be obtained; and a considerable decrease in damage caused by *A. solani* in tomato plants because bacteria of this genus commonly inhabit soils and not foliage (28). *B. Subtilis* and acetylsalicylic acid can be an alternative for the management of diseases in tomato plants, in order to reduce the use of agrochemicals, problems to the ecosystem and health.

Conclusions

B. subtilis treatment to the root showed better results than applied to the foliage. In the other hand acetylsalicylic acid was more efficient applied to the foliage than applied to the root.

The combined use of resistance inducers, such as *Bacillus subtilis* applied to the root and salicylic acid applied to the foliage, reduced the severity of *Alternaria solani* in tomato plants, and caused a significant increase in crop growth and yield.

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Acknoledgements

We thank the National Council of Science and Technology (CONACYT) known by its Spanish acronym for the Scholarship awarded for the completion of the Postgraduate Program, Maestría en Ciencias en Desarrollo Sustentable y Gestión de Riesgos, Facultad de Ingeniería, Universidad de Ciencias y Artes de Chiapas.