Colegio de Postgraduados

Severity Caused by *Fusarium oxysporum* f. sp. *lycopersici* in Tomato Varieties (*Solanum lycopersicum* L.) in Relation to Nutrition

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ABSTRACT

Objective: To evaluate nutrient solutions in *Solanum lycopersicum* as an alternative strategy for controlling Fusarium wilt.

Design/methodology/approach: Steiner nutrient solutions at 100% strength and with calcium (Ca) modifications were evaluated in four saladette tomato varieties, Bony Best (BB), 8444, 8579, and Chantico (CHAN), in order to analyze the severity index caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL). Tomato plants were inoculated with FOL race 3, and a completely randomized design with a factorial arrangement and four replicates was established. The disease severity index was assessed 30 days after inoculation using a 0-4 scale. Tukey's mean comparison test was performed (α=0.05).

Results: Treatment 1 (Ca 207 mg L^{-1}, variety BB) exhibited the lowest disease severity index. In the foliar analysis, the best treatment was also Treatment 1 (Ca 207 mg L^{-1}, variety BB), which showed the highest Ca content.

Findings/conclusions: The Bony Best variety showed the lowest disease severity index (5.37%) under a Ca concentration of 207 mg L^{-1}. Therefore, the application of this Ca dose is advisable to reduce the severity of the disease caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL).

Keywords: Nutrient solution, severity index, *Fusarium oxysporum* f. sp. *lycopersici*.

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INTRODUCTION

Tomato production (*Solanum lycopersicum* L.) has currently attained substantial economic and nutritional importance worldwide, as the global harvested area exceeds four million ha $year^{-1}$, with an average production fluctuating around 190 million t $year^{-1}$ (FAO, 2024). Tomato is native to Mexico, Peru, and Ecuador, where diverse wild varieties are found. At present, tomato is the most popular vegetable crop in the gastronomic culture

of most countries (Montes and Aguirre, 1992). The leading tomato-producing countries in the world are China, the United States, India, Italy, Egypt, Spain, the Netherlands, Mexico, and France. Mexico ranks seventh, with production exceeding 3.5 million tons and an export value of 2.613 billion dollars. The most important tomato-producing states in Mexico are Sinaloa, Baja California, San Luis Potosí, Michoacán, Morelos, Sonora, Jalisco, Nayarit, the State of Mexico, and Baja California Sur; among these, Sinaloa is the principal producing state (SIAP, 2024). However, tomato production is adversely affected by Fusarium wilt, a fungal disease caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL), which is one of the major factors limiting the successful production of this crop and may cause losses of up to 60% or even total crop failure (Tello and Lacasa, 1988).

The symptoms caused by FOL are initially observed in the root system of tomato plants, where it induces severe rot and subsequently advances through the vascular bundles of the stem toward the aerial parts, darkening them and obstructing the flow of water and nutrients. The vascular system of the root, stem, and petioles turns reddish-brown, causing vascular blockage, which in turn leads to slight yellowing and premature senescence of the lower leaves (Tello and Lacasa, 1988).

The different *Fusarium* species that cause wilt follow a similar infection pattern: they penetrate through the root and colonize the vascular system within the plant stem. However, colonization is restricted in both resistant and susceptible crops to the initial site of pathogen entry due to vessel occlusion by gel formation, callose deposits, and tyloses. In susceptible crops, colonization continues (secondary spread) when gels and callose are degraded by the pathogen's pectolytic enzymes and tylose growth is inhibited. In resistant crops, flavonoids such as catechins and their oxidation products inactivate these enzymes, and secondary spread remains limited to the initial infection sites (Gonzalez *et al.*, 2012).

Plant defense against pest and pathogen attack is highly complex, as several factors act in a coordinated manner. Two types of defenses are involved: a) constitutive defense, expressed as a normal characteristic of plant development; and b) inducible defense, which is activated when the plant comes into contact with an invading organism. This mechanism consists of a surveillance system capable of recognizing the threat, thereby triggering a signal transduction system and a response pathway, usually regulated at the transcriptional level through the expression of defense-related genes. A large number of proteins are involved in plant defense mechanisms, and these would act specifically against pathogenic organisms that attack the plant (Blanco and Aguirre, 2002).

Mineral nutrition plays an important role in disease control in plants. The use of chemicals increases the negative impact on the environment and food safety. Deficiency of any essential nutrient affects plant health and increases susceptibility to disease. Nutritionally stressed plants are more prone to disease, whereas adequately nourished plants exhibit greater disease tolerance (Sepulveda, 2006). Basic scientific research has provided specific explanations of the mechanisms through which nutrition exerts a marked influence on the incidence and severity of diseases in cultivated plants. In turn, applied research has generated findings that have laid the foundation for incorporating nutritional management into commercial integrated control schemes for the sanitary problems of various crops.

It has also been reported that imbalances between N and K, Ca and B, and among Ca, Mg, and K favor disease development, highlighting that integrated nutritional management must form part of disease management strategies (Munévar, 2004). Therefore, the aim of the present research was to determine the effect of calcium on different tomato varieties with respect to the severity caused in them by race 3 of *Fusarium oxysporum* f. sp. *lycopersici*.

MATERIALS AND METHODS

Fusarium Isolation

The isolate used was that reported by Vega *et al.* (2019), who confirmed the race of the isolates through pathogenicity and molecular tests (Table 1). The isolate is deposited in the isolate collection of the Plant Protection Laboratory of the Faculty of Agronomy at the Autonomous University of Sinaloa.

The isolate was obtained from commercial tomato (*S. lycopersicum*) crops in the state of Sinaloa, Mexico, during the 2016-2017 autumn-winter growing season.

Sowing

Seeds of four indeterminate-growth tomato varieties were sown in a substrate composed of peat and fine vermiculite using 200-cell trays, placing one seed per cell. The seedlings were maintained under suitable greenhouse conditions. The varieties used were Bony Best (BB), susceptible to the three races of FOL; 8444, resistant to the three races of FOL; 8579, resistant to the three races of FOL; and Chantico (CHAN), resistant to the three races of FOL.

Pathogenicity tests were carried out using the four tomato varieties. The root-dip method was employed, in which the roots of plants at the stage of two true leaves per genotype were washed and immersed for 10 min in a conidial suspension (1×10^5 CFU mL^{-1}) containing the isolate, and were subsequently transplanted into pots containing a sterile peat and vermiculite mixture. The suspension was obtained by collecting spores from the isolate. Forty days after inoculation, when the plants had developed four true leaves, they were harvested for the corresponding analyses (Duffy and Défago, 1999).

The nutrient solution (NS) was applied after FOL inoculation, using concentrations based on the full-strength universal Steiner solution (1966), with modifications consisting of a 15% increase in Ca concentration. The fertilizers used were potassium nitrate (KNO_3), calcium nitrate [$Ca(NO_3)_2{\cdot}4H_2O$], monopotassium phosphate (KH_2PO_4), potassium sulfate (K_2SO_4), calcium chloride ($CaCl_2$), and potassium chloride (KCl) (Table 2).

Foliar Analysis

The plants were analyzed to determine Ca concentration. The samples were placed in paper bags and dried in a forced-air oven at 70 °C±5 °C for 24 h. Once dried, the samples were ground in a mill until passing through a 1.0 mm mesh sieve. After grinding,

Table 1. Origin, code and accession number of the Genbank of *Fusarium* spp. Isolated from tomato plants.

Especies	Isolation Code	Origin	No. Genbank Access
F. oxysporum (raza3)	FOB25SINGUA	Guasave, Sinaloa	MH463538

Table 2. Treatments and factor levels.

Treatment	Fertilization	Variety
1	Steiner + 15% Ca	BB
2	Steiner + 15% Ca	8444
3	Steiner + 15% Ca	8579
4	Steiner + 15% Ca	CHAN
5	Steiner	BB
6	Steiner	8444
7	Steiner	8579
8	Steiner	CHAN

the material was homogenized and divided into 5 to 10 g portions for analysis and storage. From the homogenized sample, 2 g were placed in a crucible and introduced into a muffle furnace at 450 °C for 2 h. Subsequently, the sample was removed and allowed to cool, after which 2-3 mL of distilled water were added to moisten the ashes. The methodologies proposed by Motsara and Roy (2008) were used to calculate each element.

Severity Index and Statistical Analysis

Thirty days after inoculation, the plants were removed from the trays, labeled, and evaluated for disease severity according to the scale proposed by Marlatt and Correll (1998), with established levels from 0 to 4, where 0=0%; 1=slight necrosis (1-33%); 2=moderate necrosis and discoloration (34-66%); 3=severe necrosis and discoloration (67-100%); and 4=dead plant. A completely randomized design with a factorial arrangement and four replicates was established, resulting in a total of eight treatments. For the response variables, namely disease severity index and foliar analysis, the data were analyzed by analysis of variance and Tukey's mean comparison test (α=0.05).

RESULTS AND DISCUSSION

Foliar Analysis

The highest Ca concentration in tomato plants was found in Treatment 1 (Table 3), which combined a solution containing 207 mg L^{-1} Ca with the Bony Best (BB) variety, reaching a content of 16 mg Ca g^{-1} dry matter. This same treatment also exhibited the lowest severity index (5.37%) (Table 4), which is consistent with the findings reported by Lawrence (2007), who established that 10 to 30 mg Ca g^{-1} plant tissue are sufficient levels for optimal growth and development.

An increase in Ca content has been associated with innate immune responses during the pathogen-host interaction (Chakraborty *et al.*, 2017). Calcium acts as a structural element and promotes the activation of enzymes involved in defense against certain pathogens and physiological disorders. According to García (2001), calcium also markedly inhibits the action of polygalacturonase, since high calcium levels in the apoplast generate a greater proportion of pectates and, consequently, greater resistance to cell wall disintegration. Moreover, Ca may act as an amplifier of certain defense signals against maturity spot and enhance the speed of the plant's response to pathogen attack.

Various scientific reports have demonstrated the close relationship between disease development and calcium deficiency under water stress conditions. Fertilization practices and the behavior of calcium in relation to other soil nutrients are also factors that influence maturity spot, the incidence of which is reduced through calcium nitrate fertilization (Díaz *et al.*, 2007).

Disease Severity Index (DSI)

As shown in Table 4, Treatment 1, which combined 207 mg L-1 Ca with the BB variety, exhibited a low disease severity index of 5.37%. These results are similar to those reported by Ngosong *et al.* (2018), who found low severity levels of 10% when applying low N levels of 15 mg L^{-1}, whereas Delgado *et al.* (2016) obtained comparable results in garlic, where severity indices ranged from 2.25 to 3.5%. Likewise, Cachinero *et al.* (2002) reported a severity index of 5% in chickpea inoculated with race 5 of *Fusarium oxysporum* f. sp. *ciceris*.

Table 3. Foliar analysis of calcium in plants (mg).

Treatment	Fertilization	Variety
1	16	A
5	14	B
6	14	B
3	12	C
2	12	C
8	8	D
4	8	D
7	4	E

Treatments followed by different letters indicate a significant difference according to Tukey's test (α=0.05).

Table 4. Disease Severity Index (DSI, %).

Treatment	Fertilization	Group
7	37.50	A
4	31.56	B
8	31.56	B
3	23.25	C
2	22.50	C
6	19.12	D
5	12.75	E
1	5.37	F

Treatments followed by different letters indicate a significant difference according to Tukey's test (α=0.05).

CONCLUSIONS

The Bony Best variety exhibited the lowest disease severity index (5.37%) under a Ca concentration of 207 mg L^{-1}. Therefore, the application of this Ca dose is advisable to reduce the severity of the disease caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL).

Although the Bony Best variety is susceptible to race 3 of FOL, optimal nutrition confers upon it the capacity to defend itself against the pathogen by activating defense mechanisms. These include the increase and activation of defensive enzymes, such as phenylalanine ammonia-lyase (PAL), which plays a key role in the synthesis of secondary metabolites associated with resistance.

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