

PHYSIOLOGICAL CHANGES IN *Solanum lycopersicum* L. IN THE PRESENCE OF ROOT-KNOT NEMATODES AND SALICYLIC ACID

ALTERAÇÕES FISIOLÓGICAS EM *Solanum lycopersicum* L. NA PRESENÇA DE NEMATOIDES DAS GALHAS E ÁCIDO SALICÍLICO

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ABSTRACT: Nematodes of the genus *Meloidogyne* spp. are one of the tomato culture's main pathogens. According to their soil infestation level, physiological changes may occur. Consequently, it is extremely important to employ given products to minimize their effect. A feasible option is the salicylic acid, a phytohormone that can induce into resistance due to its ability to increase the production level of some pathogenicity proteins. Therefore, the purpose hereof was to assess the effect of salicylic acid on the physiology of tomato plants subject to different populational densities of *Meloidogyne javanica*. The design employed was one of randomized blocks, in an incomplete factorial scheme, using the Box Central Compound (BCC) matrix with five nematode populational densities (PD) (0; 5815; 20000; 34184; and 40000 eggs per plant) and five salicylic acid doses (0.0; 0.29; 1.0; 1.71; and 2.0 mM), with four repetitions and two plants per experimental patch. The gas exchanges, fluorescence, and chlorophyll levels were evaluated 45 days after the transplant and soil infestation. The data were submitted for analysis of variance through the F test and, in the significance cases, the polynomial regression analysis was performed. For stomatal conductance, a greater reduction was seen at the PD of 21755 eggs per plant, a fact that might have decreased the liquid assimilation of CO₂ and the efficiency of carboxylation. The salicylic acid (SA) affected the CO₂ liquid assimilation and the efficiency of carboxylation. The infestation of *M. javanica* in tomato plants negatively affected the gas exchange and the chlorophyll levels because the SA application did not mitigate the negative effect thereof.

KEYWORDS: Chlorophyll. Gas exchange. *Meloidogyne javanica*. Tomato plant.

INTRODUCTION

The tomato plant (*Solanum lycopersicum* L.) is a vegetable of great socioeconomic importance, being part of the nourishment of many Brazilians (SCHWARZ et al., 2013). In Brazil, the culture was introduced by European immigrants at the end of the 19th century, where it later became the second greatest vegetable in economic importance (ZHOU et al., 2017).

Among the phytosanitary problems that are harmful to this culture, the meloidoginosis, a disease caused by nematodes of the genus *Meloidogyne* spp. (SOUSA et al., 2010) can be stressed. The phytoparasite nematodes generate loss both in the production and in the quality of the fruits, reaching losses of even 100% due to the high levels of infestation at the beginning of the plantation (OLIVEIRA; ROSA, 2014).

The control methods employed to deal with these pathogens are both hard and expensive, especially when nematicides are employed, as the best control method is to avoid the establishment of nematodes in the area (VAZ et al., 2011). In this sense, the search for products that mitigate such effects is of great importance. Before such a need, the salicylic acid (SA), a hormone responsible for regulating several vegetable growth and development features, which can make the plants respond to biotic and abiotic stresses (FU et al., 2012), can become a feasible alternative.

The SA is synthesized by the plants in response to the attack of several kinds of phytopathogens, being essential for the establishment of a systemic acquired resistance (SAR) (MORADI; REZVANI, 2012). Positive effects of SA are already reported in some studies (CAMPOS et al., 2014; SILLERO et al., 2012).

In this context, there are few pieces of information regarding the SA's effect on the physiological behavior of tomato plants, especially in a soil infested by nematodes. Therefore, the purpose hereof was to assess the effect of salicylic acid in the features of gas exchange, chlorophyll levels, and fluorescence parameters of tomato plants (*Solanum lycopersicum* L.) subject to different populational densities of *Meloidogyne javanica*.

MATERIAL AND METHODS

The research was conducted in a vegetation house of the Plant Breeding and Environmental Sciences Department of the Universidade Federal da Paraíba (UFPB), located at the city of Areia, Paraíba.

To obtain the inoculum, a population of *M. javanica* was kept and multiplied in tomato plants (*Solanum lycopersicum* L. cv. Santa Clara), which were cultivated in vases with a capacity of 2 dm³ Dystrophic Regolithic NEOSSOLO soil and sand (2:1) and kept in a vegetation house for the period of 70 days.

The nematode extraction was performed according to the methodology suggested by Hussey and Barker (1973) and adapted by Bonetti and Ferraz (1981). The tomato plants' aerial part was separated from the root system, after which the roots

were washed, selected in parts and shredded in blender in a sodium hypochlorite (NaClO) solution at 0.5% at low rotation for 20 seconds. The solution was sieved in sieves of 200 and 500 mesh (openings of 74 and 22 mm, respectively). The contents of the 500 mesh sieve were washed with water to eliminate every trace of the sodium hypochlorite, gathered into a beaker, after which the amount of eggs was quantified in an optical microscope. Then, the soil infestation was performed according to the treatments.

The tomato cultivar employed was the Santa Cruz Kada (Paulista) (Isla[®]). Its seedlings were produced in polyethylene trays, using a commercial substrate (Basaplant[®]). Three seeds were planted by cell. The excess of seedlings was removed after the emergence thereof. Twenty-five days after plantation (DAP), the seedlings were transplanted into vases with a capacity of 5 dm³, filled with a substrate made with a mixture of soil, sand, and cattle manure in the proportion of 3:1:1, previously sterilized in autoclave at a temperature 120 °C, 1 atm of steam pressure for 2 hours. The irrigations were performed whenever necessary, keeping the moisture of the soil close to the field capacity. A substrate sample was collected for fertility analysis, which was performed in the Soil Analysis Laboratory of the Universidade Federal da Paraíba, Agricultural Science Center, Campus II (Table 1).

Table 1. Chemical features of the components of the substrate employed in the experiment. Mossoró-RN, Brazil, 2020.

pH	P	K ⁺	Na ⁺	H ⁺ +Al ⁺³	Al ⁺³	Ca ⁺²	Mg ⁺²	BS	CEC	O.M.
	--- mg kg ⁻³ ---			----- cmol _c dm ⁻³ -----						g kg ⁻¹
7.8	85.55	693.60	0.23	0.00	0.00	2.91	1.59	6.50	6.50	22.21

BS: Base sum; CEC: cation-exchange capacity; O.M.: Organic matter. UFERSA/DCAF, Mossoró, RN.

Distilled water was employed to prepare the doses of salicylic acid (SA). After the transplant, three applications were performed in intervals of 15 days, when the tomato plant's leaves were pulverized on both sides until they were completely wet, which was performed after 4 p.m.

The experimental design was made in randomized blocks, in an incomplete factorial scheme, with five populational densities of *Meloidogyne javanica* (0; 5815; 20000; 34184; and 40000 eggs per plant) and five doses of salicylic acid (0.0; 0.29; 1.0; 1.71; and 2.0 mM), with four repetitions, and two plants per experimental patch, resulting in nine combinations (treatments),

generated through the Box Central Compound matrix.

The evaluations were performed 45 days after plantation (DAP) and soil infestation with the nematodes. To establish the gas exchange, an infrared gas analyzer - IRGA (model LI-6400XT, Li-COR[®], Nebraska, USA) was employed with an air flow of 300 μmol s⁻¹ and an attached light source of 1200 μmol m⁻² s⁻¹, whose measurements were taken at 9 and 10 a.m. The following variables were measured: CO₂ liquid assimilation (*A*) (μmol CO₂ m⁻² s⁻¹), stomatal conductance (*gs*) (mol H₂O m⁻² s⁻¹), CO₂ concentration in the intercellular spaces (*Ci*) (μmol CO₂ m⁻² s⁻¹) transpiration (*E*) (mmol H₂O m⁻²

s^{-1}), water use efficiency (WUE= A/E), water use intrinsic efficiency (WUEi= A/g_s), carboxylation instant efficiency (iCE= A/C_i), and leaf temperature (LT) ($^{\circ}C$).

To evaluate the variable chlorophyll fluorescence, a modulated fluorometer (Sciences Inc. - Model OS-30p, Hudson, USA) was employed. Leaf calipers were inserted for 30 minutes before the readings to adapt the leaves for the dark. The initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence ($F_v=F_m-F_0$), ratio F_v/F_0 , and quantum yield of the photosystem II (F_v/F_m) were measured. The measurement of the chlorophyll a, b, and total levels were performed through a non-destructive method, employing a portable chlorophyll meter (ClorofiLOG[®], model CFL 1030, Porto Alegre, RS), whose values were given in Falker chlorophyll index (FCI).

The data were submitted for analysis of variance through the F test up to a probability of 5%. In the cases of significance, a polynomial regression analysis was performed, using the statistical program SAS[®] (CODY, 2015).

RESULTS AND DISCUSSION

There was no significant interaction between the evaluated features, populational densities (PDs), and salicylic acid (SA). Based on the analysis of variance, it is possible to state that there was a PD significant effect only for stomatal conductance, CO_2 liquid assimilation, leaf temperature, and carboxylation efficiency. As for the SA, only the CO_2 liquid assimilation and the carboxylation efficiency were significantly affected (Table 2).

Table 2. Summary of the analysis of variance, by the values of mean square, for stomatal conductance (g_s), CO_2 liquid assimilation (A), CO_2 internal concentration (C_i), transpiration (E), leaf temperature (LT), water use intrinsic efficiency (WUEi), water use efficiency (WUE), and carboxylation instant efficiency (iCE) in tomato plants (*Solanum lycopersicum* L.) in different populational densities of *Meloidogyne javanica* and salicylic acid. Mossoró-RN, Brazil, 2020.

SV	DF	MS			
		g_s	A	C_i	E
Block	3	0.0050 ^{ns}	1.2934 ^{ns}	1762.6050**	0.5097 ^{ns}
SA	1	0.0069 ^{ns}	0.1987 ^{ns}	0.0758 ^{ns}	2.2941 ^{ns}
PD	1	0.0035 ^{ns}	13.0767**	6.0836 ^{ns}	0.7448 ^{ns}
SA*SA	1	0.0071 ^{ns}	26.2312**	533.6495 ^{ns}	1.4226 ^{ns}
PD*PD	1	0.0088*	14.9390**	216.3930 ^{ns}	0.9606 ^{ns}
SA*PD	1	0.0019 ^{ns}	3.3666 ^{ns}	104.9769 ^{ns}	0.7637 ^{ns}
Residue	24	0.0019	1.5703	174.1208	0.5512
CV (%)		29.27	15.8	4.59	21.17
		Tleaf	iWUE	WUE	iCE
Block	3	25.3674**	1.011.7620 ^{ns}	0.2628 ^{ns}	2.0920E-05 ^{ns}
AS	1	0.4867 ^{ns}	2.759.9209 ^{ns}	1.5854 ^{ns}	4.3400E-06 ^{ns}
PD	1	0.3626 ^{ns}	39.3199 ^{ns}	0.2733 ^{ns}	1.8787E-04*
SA*SA	1	0.0379 ^{ns}	288.1679 ^{ns}	0.7121 ^{ns}	4.3991E-04**
PD*PD	1	1.7899**	134.3402 ^{ns}	0.5513 ^{ns}	1.6101E-04*
SA*PD	1	0.0168 ^{ns}	5.2544 ^{ns}	0.0023 ^{ns}	7.2090E-05 ^{ns}
Residue	24	0.1565	731.5564	0.4867	2.4430E-05
CV (%)		1.33	39.64	28.21	17.45

SV= source of variation; MS= medium square; DF= degree of freedom; SA= salicylic acid; PD= populational densities; CV%= coefficient of variation; ** and * = significant at 1 and 5% probability, by the F test.

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For g_s , it was registered a greater PD reduction for 21755 eggs per plant (0.12 mol of H_2O $m^{-2} s^{-1}$), with a decrease of 36.84%. It is important to stress that in the PDs above the aforementioned, the g_s presented the tendency to increase, exhibiting a

decrease of only 5.26% in the PD of 40000 eggs in comparison with the control treatment (Figure 1 A). This g_s decrease can be the result of a resistance mechanism of the plant subjected to light stress, therefore saving water, because there was a lower

stomatal limitation with the PD increase in comparison with the control treatment (CHAVES et al., 2009). Gs increase events were also reported by Tahery (2012) in some cultivars of *Hibiscus*

cannabinus infected with *M. incognita* 60 days after inoculation.

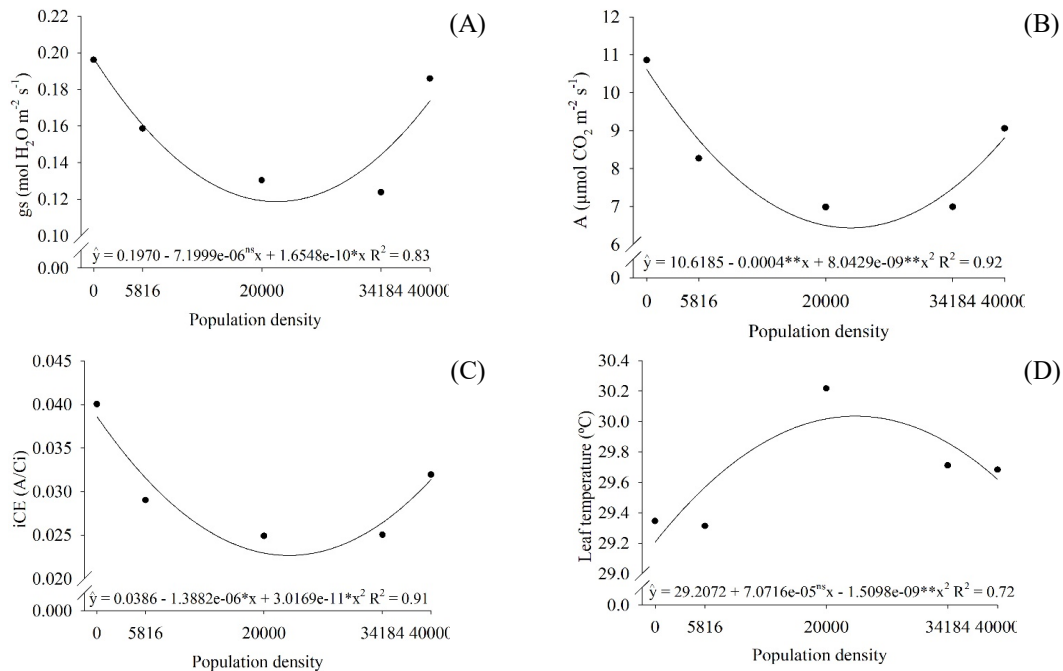


Figure 1. A) Stomatal conductance, B) CO₂ liquid assimilation, C) carboxylation efficiency, and D) leaf temperature in tomato plants (*Solanum lycopersicum* L.) under different populational densities of *Meloidogyne javanica*.

As for the CO₂ liquid assimilation (*A*), a lower level (5.65 μmol of CO₂ m⁻² s⁻¹) was registered for the PD of 24867 eggs, which was directly proportional to the inoculum's populational density (Figure 1 B). This drop registered in *A* can be related to stomatal limitation, resulting in the decrease of absorption and of the CO₂ amount in the active area of Rubisco (TAIZ et al., 2017). Tahery (2012) registered results different from the ones hereof in *Hibiscus* L. plants.

As for the increase of *A* with the growth of the PD, it might have happened due to a greater energy demand to trigger resistance mechanisms. Another explanation could be that this photosynthesis increase would result in a greater amount of photosynthetic products sent to the root system to feed the nematodes (STRAJNAR et al., 2012). Different results were registered by Souza (2008) in coffee plants in the presence of nematodes, in which there was no significant difference for photosynthesis and stomatal conductance.

For iCE, the greatest decrease was noticed in the PD of 23007 eggs, with a reduction of 50% in comparison with the control (Figure 1 C), proving that there was a reduction in the amount of CO₂

fixed by Rubisco in the aforementioned PD. Schock et al. (2014) link such decrease in the carboxylation efficiency to the stomatal limitation, which reduces the CO₂ input to the cells.

The populational density of 23419 eggs generated a greater LT (30.0 °C) (Figure 1 D). It can be noticed that the LT is inversely proportional to the one registered for gs, probably in response to the stomatal closure, which reduces the transpiration level and consequently the water flow in the plant (ELLI et al., 2013).

As for the effect of the salicylic acid (SA), it was noticed that the concentrations employed significantly affected only the CO₂ liquid assimilation and the carboxylation efficiency in tomato plants (Figure 2 A). It can be noticed that the concentration of 0.96 mM of SA generated greater *A* (8.90 μmol CO₂ m⁻² s⁻¹), with an increase of 25.17% in comparison with control (Figure 2 A).

This *A* increase can be related to the fact that the SA is a phytohormone, which, when exogenously applied can induce several physiological process, including the photosynthetic level (AGOSTINI; MACHADO NETO; CUSTÓDIO, 2013). Photosynthesis increase results were registered by Ghasemzadeh and Jaafar (2013)

in ginger plants (*Zingiber officinale* L.) and in corn (*Zea mays* L.) by Hayat et al. (2010) under SA

application.

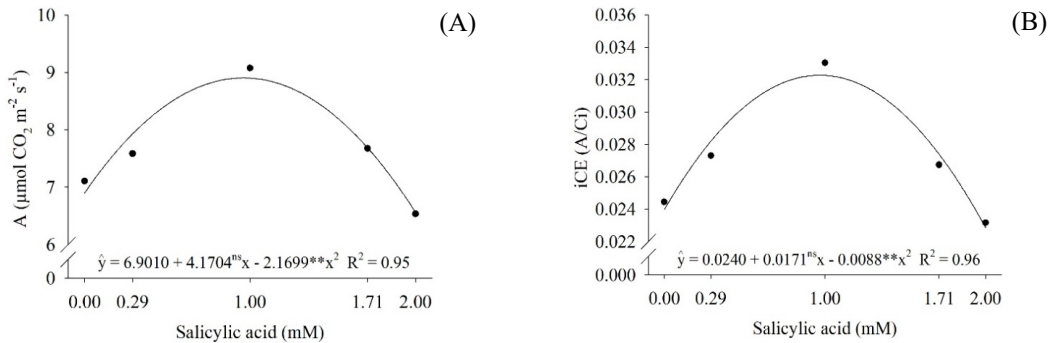


Figure 2. A) CO₂ liquid assimilation and B) carboxylation instant efficiency in tomato plants (*Solanum lycopersicum* L.) under salicylic acid application.

For iCE, the best performance was registered at the dosage of 0.97 mM, with an increase of 50% (Figure 2 B). Ferraz et al. (2012) reported that the CO₂ assimilation increase implies a greater carboxylation efficiency because a greater amount of CO₂ is being fixed by Rubisco, a fact that we can notice herein.

According to the result of the analysis of variance, when the isolated effect of the factors is observed, it is possible to notice that only the populational density (PD) factor significantly affected the chlorophyll levels, presenting no difference for all other variables. Additionally, the salicylic acid had no effect (Table 3).

Table 3. Summary of the analysis of variance by the mean square values for chlorophyll *a* (Cl_a), *b* (Cl_b), total chlorophyll (Cl_t), chlorophyll *a/b* ratio (Cl_{a/b}), initial (F₀), variable (F_v), and maximum fluorescence (F_m), PSII quantum efficiency (F_v/F_m), and F_v/F₀ ratio in tomato plants (*Solanum lycopersicum* L.) under different populational densities of *Meloidogyne javanica* and salicylic acid. Mossoró-RN, Brazil, 2020.

SV	DF	MS				
		Cl _a	Cl _b	tCl	Cl _a /Cl _b	
Block	3	5.4813 ^{ns}	1.6719 ^{ns}	9.8543 ^{ns}	0.3059 ^{ns}	
SA	1	10.0331 ^{ns}	0.7989 ^{ns}	16.4942 ^{ns}	0.0301 ^{ns}	
PD	1	27.4654*	5.0502*	56.0702*	0.3682 ^{ns}	
SA*SA	1	0.5957 ^{ns}	1.0450 ^{ns}	3.2186 ^{ns}	0.0496 ^{ns}	
PD*PD	1	1.3129 ^{ns}	0.0871 ^{ns}	2.0762 ^{ns}	0.0292 ^{ns}	
SA*PD	1	2.2500 ^{ns}	0.4727 ^{ns}	4.7852 ^{ns}	0.0429 ^{ns}	
Residue	24	3.8877	1.0995	8.2703	0.1554	
CV (%)		7.28	15.93	8.54	9.23	
		F ₀	F _m	F _v	F _v /F _m	F _v /F ₀
Block	3	285.1481 ^{ns}	645.6111 ^{ns}	1.727.2778 ^{ns}	0.0032 ^{ns}	0.6552**
SA	1	123.4819 ^{ns}	4.262.7896 ^{ns}	2.935.2339 ^{ns}	2.6900E-06 ^{ns}	0.0057 ^{ns}
PD	1	23.6689 ^{ns}	398.6297 ^{ns}	228.0295 ^{ns}	0.0002 ^{ns}	0.0478 ^{ns}
SA*SA	1	66.7795 ^{ns}	222.0512 ^{ns}	532.3751 ^{ns}	0.0019 ^{ns}	0.2220 ^{ns}
PD*PD	1	505.5623 ^{ns}	706.2515 ^{ns}	16.7337 ^{ns}	0.0010 ^{ns}	0.2940 ^{ns}
SA*PD	1	50.6250 ^{ns}	9.7656 ^{ns}	28.8906 ^{ns}	0.0007 ^{ns}	0.0449 ^{ns}
Residue	24	168.6742	1.297.9184	1.373.3924	0.0006	0.1131
CV (%)		9.89	7.14	9.93	3.38	11.65

SV= source of variation; MS= medium square; DF= degree of freedom; SA= salicylic acid; PD= populational densities; CV%= coefficient of variation; ** and * = significant at 1 and 5% probability, by the F test.

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The levels of chlorophyll *a* (Cl_a), chlorophyll *b* (Cl_b) and total chlorophyll (Cl_t) were affected by the populational densities of *M. javanica*, adjusting themselves to a decreasing linear effect (Figure 3). For Cl_a, a decrease of 9.66% was registered (Figure 3 A), Cl_b decreased 16.55% (Figure 3 B), and Cl_t presented a decreased of 11.05% (Figure 3 C). These decrease events were

registered for the PD of 40000 eggs per plant, respectively, in comparison with the control treatment. It might have happened because the increase of the populational density generated an increase in the levels of the chlorophyllase enzyme, whose function is to degrade this photosynthetic pigment's molecules (FREIRE et al., 2013).

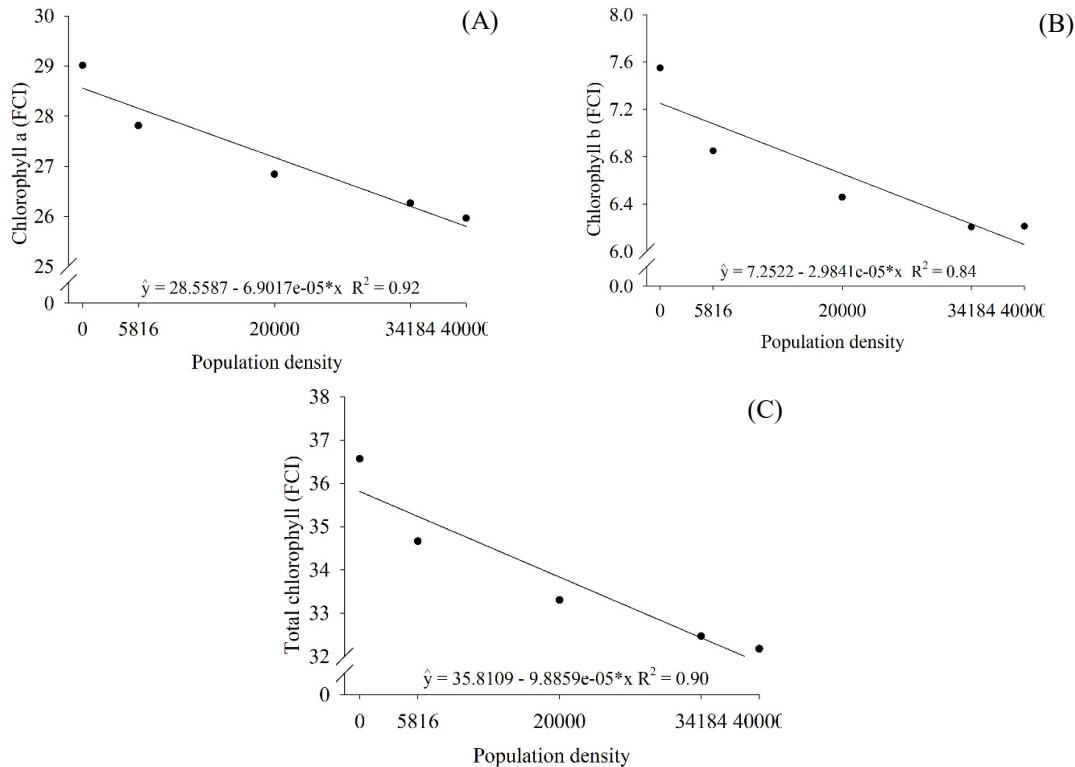


Figure 3. A) Chlorophyll *a*, B) chlorophyll *b* and C) *total* chlorophyll in tomato plant (*Solanum lycopersicum* L.) under different populational densities of *Meloidogyne javanica*.

The incident light absorption made by plants attacked by phytopathogens undergoes changes in the interval of the visible and infrared region, a fact caused by the reduction in the level of chlorophylls, changes in other pigments, or in the leaf's structure itself (NAUE et al., 2010). In cotton plants (*Gossypium hirsutum* L.), the chlorophyll levels were directly proportional to the level of the *M. incognita* inoculum (ABRÃO; MAZZAFERA, 2001), results that are different from the ones hereof. On the other hand, Asmus and Ferraz (2001) reported that the chlorophyll level suffered little influence of *M. javanica* in soy plants (*Glycine max* L.). However, Ahmed et al. (2009) noticed that the chlorophyll levels decreased in bean plants (*Phaseolus vulgaris* L.) infected by *M. javanica*, a result that agrees with this study.

The mean values for F_0 , F_m , and F_v indicate that all reaction centers are open and reached their maximum capacity of photochemical reactions

because there was no fluorescence change, which leads us to attest that there was no compromise in the photosynthetic process. The fact that there was no change in F_v/F_m between the periods proves that the parasitism by *M. javanica* did not interfere in the electron transportation by PSII (ASMUS; FERRAZ, 2001).

These results agree with the ones found by Figueiredo et al. (2018) in eggplants (*Solanum melongena* L.), in which, according to the same authors, this behavior can be a defense mechanism of the plant, which prevents the nematodes from causing physiological and biochemical disorders. Silva et al. (2015) state that, under F_v/F_m values between 0.75 and 0.85 electrons quantum⁻¹, the plants perform their physiological activities properly. The values found herein are quite close to this interval, proving that the treatments generated few changes in the plants' physiological apparatus.

As for SA, this phytohormone affects the plants' functions according to the dosage. In other words, the plant functions can be induced or inhibited according to the low or high concentrations of SA, respectively, a fact that probably affected the data found herein (KHAN et al., 2015). This phytohormone induces a wide set of metabolic and physiological responses in the plants, such as resistance to diseases, ethylene biosynthesis, and oxidative stress mitigation (ASGHARI; AGHDAM, 2010), something that was not seen in the aforementioned parameters.

CONCLUSIONS

The infestation by *M. javanica* in tomato plants negatively affects the gas exchange and the chlorophyll levels.

The PD of 24867 eggs is the one that most affects tomato photosynthesis.

The dosage of 0.96 and 0.97 mM of SA results in a greater CO₂ liquid assimilation and carboxylation instant efficiency, respectively.

The application of salicylic acid does not mitigate the negative effects of *M. javanica* in tomato plants.

ACKNOWLEDGEMENTS

The authors thank the Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support of this research.

RESUMO: Os nematoides do gênero *Meloidogyne* spp. são um dos principais patógenos na cultura do tomate, podendo causar alterações fisiológicas em função do nível de infestação do solo. O uso de produtos que minimize seus efeitos é de suma importância, nesse sentido, o ácido salicílico pode ser uma alternativa viável, visto que, esse fitormônio pode induzir a resistência devido à capacidade de proporcionar um aumento na produção de algumas proteínas de patogenicidade. Com isso, objetivou-se avaliar o efeito do ácido salicílico na fisiologia do tomateiro submetido a diferentes densidades populacionais de *Meloidogyne javanica*. O delineamento utilizado foi o de blocos casualizados, em esquema fatorial incompleto, utilizando-se a matriz Composto Central de Box (CCB) com cinco densidades populacionais (DP) de nematoides (0; 5815; 20000; 34184 e 40000 ovos por planta) e cinco doses de ácido salicílico (0,0; 0,29; 1,0; 1,71 e 2,0 mM), com quatro repetições e duas plantas por parcela experimental. Foram avaliadas as trocas gasosas, fluorescências e índices de clorofilas aos 45 dias após o transplante e infestação do solo. Os dados foram submetidos à análise de variância pelo teste F e nos casos de significância foi realizada análise de regressão polinomial. Para a condutância estomática, observou-se uma maior redução na DP de 21755 ovos por planta, fato que pode ter ocasionado reduções na assimilação líquida de CO₂ e na eficiência de carboxilação. O ácido salicílico (AS) influenciou na assimilação líquida de CO₂ e eficiência de carboxilação. A infestação por *M. javanica* em plantas de tomateiro influenciou negativamente nas trocas gasosas e nos teores de clorofila, sendo que a aplicação do AS não atenuou os efeitos negativos desses patógenos.

PALAVRAS-CHAVE: Clorofila. *Meloidogyne javanica*. Tomateiro. Trocas gasosas.

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