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Gas exchange and chlorophyll content in tomato grown under different organic fertilizers and biofertilizer doses

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The correct management of organic fertilization has been shown as an extremely viable alternative in the production of vegetables, providing high yields concomitant to the reduction of synthetic fertilizers. The improvement of the production can be interpreted by the physiological behavior, favored by the organic fertilization with the supply of nutrients. Thus, an experiment was carried out to evaluate the gas exchange and Soil-Plant Analyses Development (SPAD) chlorophyll content in tomato plants according to types of organic fertilizers and biofertilizer doses. The experimental design was completely randomized with treatments distributed in factorial arrangement (3 x 5), referring to organic fertilizer types (T1: earthworm humus; T2: goat manure and T3: cattle manure) and biofertilizer concentrations (600, 800, 1000, 1200 and 1400 ml), with four replications. The gas exchange and the SPAD chlorophyll content in tomato plants depend on the type of organic fertilizer and the concentration of biofertilizer. It was possible to observe that increasing doses of biofertilizer in the substrate with low organic concentration increase the gas exchange in tomato plants, while high doses together with more concentrated organic fertilizers reduce these characteristics.

Key words: Alternative fertilizer, *Lycopersicon esculentum*, photosynthesis, organic fertilizer.

INTRODUCTION

The tomato (*Lycopersicon esculentum* Mill.) is a plant of great economic importance, and is acknowledged to be among the most consumed vegetables, due to the high nutritional value of its fruits, rich in antioxidants (Filgueira, 2008). The fruit yield and quality are influenced by several factors, such as growth conditions, which include plant nutrition (Oliveira et al., 2014; França et al., 2017; Ersahin et al., 2017).

Among the tomato cropping systems, organic fertilization needs to be highlighted because it improves the soil and the plants, resulting in production reductions, since the producer can replace the commercial substrate with inputs found on the site (Oliveira et al., 2013a). In addition, the demand for organic products has expanded in recent times, opening possibilities of adding value to products (Santos et al., 2013).

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The tomato crop responds satisfactorily to organic fertilization, however, factors such as type and quantity of fertilizers are determinant in improving plant growth and adequate development (Mueller et al., 2013, França et al., 2017).

The types of fertilizers seem to vary greatly in their influence on plant growth, probably due to the composition and, consequently, to the number of nutrients offered (Oliveira et al., 2014). However, different organic compounds such as earthworm humus, goat manure and cattle have positively influenced the plants when used in an adequate amount. The worm humus can be an excellent alternative to increase the commercial substrate in the organic production system, since mineral fertilizers are not permitted (Oliveira et al., 2013a). Already, it is known that the goat manure has better structure than other types, allowing better aeration, and therefore it ferments quickly and can be availed after a less period of decomposition (Cavalcante et al., 2010), while the cattle manure brings benefit to the plants, because it provides organic nitrogen accumulation in the soil (Oliveira et al., 2014; Santana et al., 2017). In addition, the combined use of organic fertilizer and biofertilizer can be a good alternative for organic fertilization in tomato, because besides providing more quantity may promote more displacement of nutrients to the roots.

The response of organic fertilization has been positively verified as a viable alternative in the production of different vegetables, such as tomato (Yanar et al., 2011; Mueller et al., 2013; França et al., 2017), okra (Oliveira et al., 2013b; Gomes et al., 2017), sugar beet (Gondim et al., 2015), eggplant (Santos et al., 2013) and gherkin (Oliveira et al., 2014).

However, although the effects of organic fertilizer on the growth and production of several vegetable species have been widely reported, studies evaluating the physiological responses as gas exchange and chlorophyll content are not common. Some researchers investigated the gas exchange in tomato plants cultivated under drought stress (Zhu et al., 2012) and salinity (Horchani et al., 2010), which observed changes as to the cultivation conditions, demonstrating the importance of evaluating also under organic cultivation. Photosynthesis is the main physiological process that is affected by changes in growth conditions, can be evaluated by gas exchange measurements based on CO_2 assimilation, as well as chlorophyll content, based on the SPAD index (Santos et al., 2010; Zhu et al., 2012). Towards the exposed, the objective of this work was to evaluate the influence of different types of organic fertilizers and doses of biofertilizer in the gas exchange and SPAD chlorophyll index in tomato plants.

MATERIALS AND METHODS

The experiment was conducted between July and September 2016, in a protected environment, in the State University of Paraíba, Campus IV, Paraíba, Brazil. The site is situated in the coordinates 6°20'38" S 37°44'48' W and 275 m of altitude. The climate of the region according to the classification of Koppen is of type BSWh, that is, hot and dry, with two distinct seasons: one rainy with irregular precipitation and another without precipitation. The maximum, average and minimum internal temperature in the protected environment was around 42, 34 and 19°C, respectively, with relative air humidity varying from 35 to 52% between the months of conduction of the experiment. The design was completely randomized, with factorial arrangement 3 × 5, referring to three types of fertilizer: T1 = 20% earthworm humus + 80% soil; T2 = 30% goat manure + 70% soil; T3 = 40% cattle manure + 60% soil, and five biofertilizer doses: 600, 800, 1000, 1200 and 1400 ml, with 4 replications, totaling 60 experimental units. Doses of biofertilizer were split and applied two times, the first being the 25 DAT (days after transplanting), and the second at 35 DAT. For the substrate composition, was used a eutrophic Flubic Neosol (EMBRAPA, 2013), plus percentages of earthworm humus, goat manure and cattle. After collecting soil samples in the superficial layer (0-20 cm), the chemical and physical characteristics were determined (Table 1). The chemical characteristics of earthworm humus and goat and cattle manure were also determined (Table 2), according to the methodology proposed by EMBRAPA (2011). The preparation of the bovine biofertilizer was carried out according to Santos et al. (2014), lasting approximately 35 days for complete fermentation, and obtaining the liquid compound (Table 3).

The seeds were sown in trays containing 128 cells, adding three seeds per cell filled with commercial substrate Basaplant®. The thinning was performed when the seedlings presented a definitive pair of leaves, approximately 10 days after sowing (DAS), leaving the most vigorous. The seedlings were irrigated daily until transplanting, which was carried out in plastic pots filled with 7 kg of soil + substrate corresponding to each treatment. The tomato variety used was I-5300 (cv. Santa Clara), widespread in Brazil, whose seed germination was 96% and purity 99%. The irrigation of the plants was performed with a uniform volume of water, as a function of crop evapotranspiration. The volume applied (Va) per container was obtained by the difference between the average weight of the container in the condition of 100% of the available water (Paw) and the average weight of the containers in the current condition before irrigation. The weight of the pots with soil field capacity (100% water available) was determined by saturating the soil and submitting to drainage; when the volume was decreasing, the pots were weighed and the difference in weight in relation to the vessels (Paw) was considered as evapotranspirated water, whose volume was restored.

At 45 days after transplanting (DAT) measurements of the gas exchange were made on the third leaf from the apex, with the help of the portable infrared carbon analyzer (IRGA), model LCPro+ Portable Photosynthesis System® (ADC BioScientific Limited, UK), with temperature adjusted to 25 °C, irradiation of 1800 μmol photons m^{-2} $s^{-1}$and flow of air 200 ml min^{-1}. The physiological variables evaluated were internal CO_2 concentration (Ci - μmol mol^{-1}), stomatal conductance (gs - mol H_2O m^{-2} s^{-1}), transpiration (E - mmol H_2O m^{-2} s^{-1}) and photosynthesis (A - μmol CO_2 m^{-2} s^{-1}). The instantaneous efficiency of water use (WUE) was obtained from the relationship between photosynthesis rate (A) and transpiration (E), and the instantaneous efficiency of carboxylation (EiC) between photosynthesis rate (A) and the internal concentration of carbon (Ci).

The SPAD index readings were performed on the same leaf used in the gas exchanges, using the SPAD-502 Chlorophyll Meter. Three readings per plant were collected, aiming at greater representativeness. Then, the average per plant was calculated, on the equipment itself. Data were submitted to analysis of variance, at a significance level of 5% probability. When significant, the regression analysis was performed for the unfolding of the s

Table 1. Chemical and physical characteristics of the soil used in the experiment.

Chemical	Values	Physical	Values
Hydrogen ion potential (H_2O) (1:2.5)	6.7	Sand (g kg^{-1})	640.00
Calcium ($cmol_c dm^{-3}$)	1.49	Silt (g kg^{-1})	206.00
Magnesium ($cmol_c$ dm^{-3})	0.54	Clay (g kg^{-1})	154.00
Sodium ($cmol_c dm^{-3}$)	0.10	Textural classification	Sandy frank
Potassium ($cmol_c dm^{-3}$)	1.72	Total Density (g dm^{-3})	1.54
Sum of bases ($cmol_c dm^{-3}$)	3.85	Density of particles (g dm^{-3})	2.68
Hydrogen + Aluminum ($cmol_c dm^{-3}$)	0.00	Total porosity (%)	42.54
Cation exchange of capacity ($cmol_c dm^{-3}$)	3.85	Field capacity (g kg^{-1})	146.9
Bases Saturation (V %)	100	Permanent wilting point (g kg^{-1})	76.60
Qualitative calcium carbonate	Wanting	Water available (g kg^{-1})	70.3
Organic carbon (%)	0.67		
Organic matter (%)	1.2		
Nitrogen (%)	0.07		
Assimilable phosphorus (mg dm^{-3})	16.83		

Table 2. Chemical characteristics of the organic fertilizers used: earthworm humus, goat manure and bovine.

Mineral nutrients												
N	P	K	Ca	Mg	Na	Zn	Cu	Fe	Mn	MO	CO	C/N
.... g kg^{-1}				.... mg kg^{-1}						.... g kg^{-1}		
Earthworm húmus												
11.8	0.4	4.1	14.2	4.0	-	84	10.8	-	237	-	-	-
Goat manure												
21.9	5.0	3.10	38.2	4.5	7.0	55	33	9567	370	433.0	340.5	15:1
Cattle manure												
12.7	2.5	16.7	15.5	4.0	5.59	60	22	8550	325	396.0	229.7	18:1

MO: organic matter; CO: organic carbon; C/N: carbon nitrogen ratio.

Table 3. Chemical characteristics of the biofertilizer used in the experiment.

Chemical characteristics	Values
Hydrogen ion potential	7.10
Electric conductivity - dS m^{-1}	5.13
Cations - $cmol_c$ L^{-1}	---
Calcium	1.75
Magnesium	1.20
Sodium	1.34
Potassium	0.91
Anions - $cmol_c$ L^{-1}	---
Chloride	2.53
Carbonate	0.33
Bicarbonate	1.56
Sulfate	0.79

biofertilizer doses, and the Tukey test for comparison of the substrates, using SISVAR.

RESULTS AND DISCUSSION

There was a significant effect of the interaction between types of fertilizer and doses of biofertilizer for stomatal conductance, transpiration, photosynthesis and instantaneous carboxylation efficiency (Table 4). As for the isolated factors, types of fertilizer affected stomatal conductance, internal CO_2 concentration and SPAD, while only the internal CO_2 concentration had no affected by the biofertilizer dose. Stomatal conductance increased by 275% in T1 fertilizer, with the increase of biofertilizer doses until 1400 ml. In T2 and T3, gs reached a maximum of 0.066 and 0.103 mol H_2O m^{-2} s^{-1} at the estimated doses of 1100 and 1225 ml $plant^{-1}$, respectively (Figure 1A). Similarly, transpiration increased 56.7% in the T1 fertilizer, with the increase of biofertilizer doses until 1400 mL, while in T2 and T3 transpiration reached a

Table 4. Summary of variance analysis (values of F) for stomatal conductance (gs), transpiration (E), photosynthetic rate (A), internal CO_2 concentration (Ci), water use efficiency (WUE), instantaneous carboxylation efficiency (EiC), foliar temperature (Tleaf), chlorophyll content (SPAD) and root-shoot ratio (R-SR) in tomato plants grown under different organic fertilizers and biofertilizer doses.

Sources of variation	gs	E	A	Ci	WUE	EiC	Tleaf	SPAD	R-SR
Types of Fertilizer (T)	3.17*	2.32^{ns}	0.49^{ns}	6.37**	2.44^{ns}	2.13^{ns}	0.09^{ns}	16.73**	61.83**
Biofertilizer doses (D)	15.51**	15.95**	14.97**	1.34^{ns}	2.58*	12.93**	9.56**	9.70**	11.00**
Interaction (TxD)	4.74**	4.00**	2.87*	0.96^{ns}	0.77^{ns}	2.60*	0.57^{ns}	1.58^{ns}	28.96**

* and ** Significance level of 5 and 1%, respectively, whereas, ns no statistical differences.

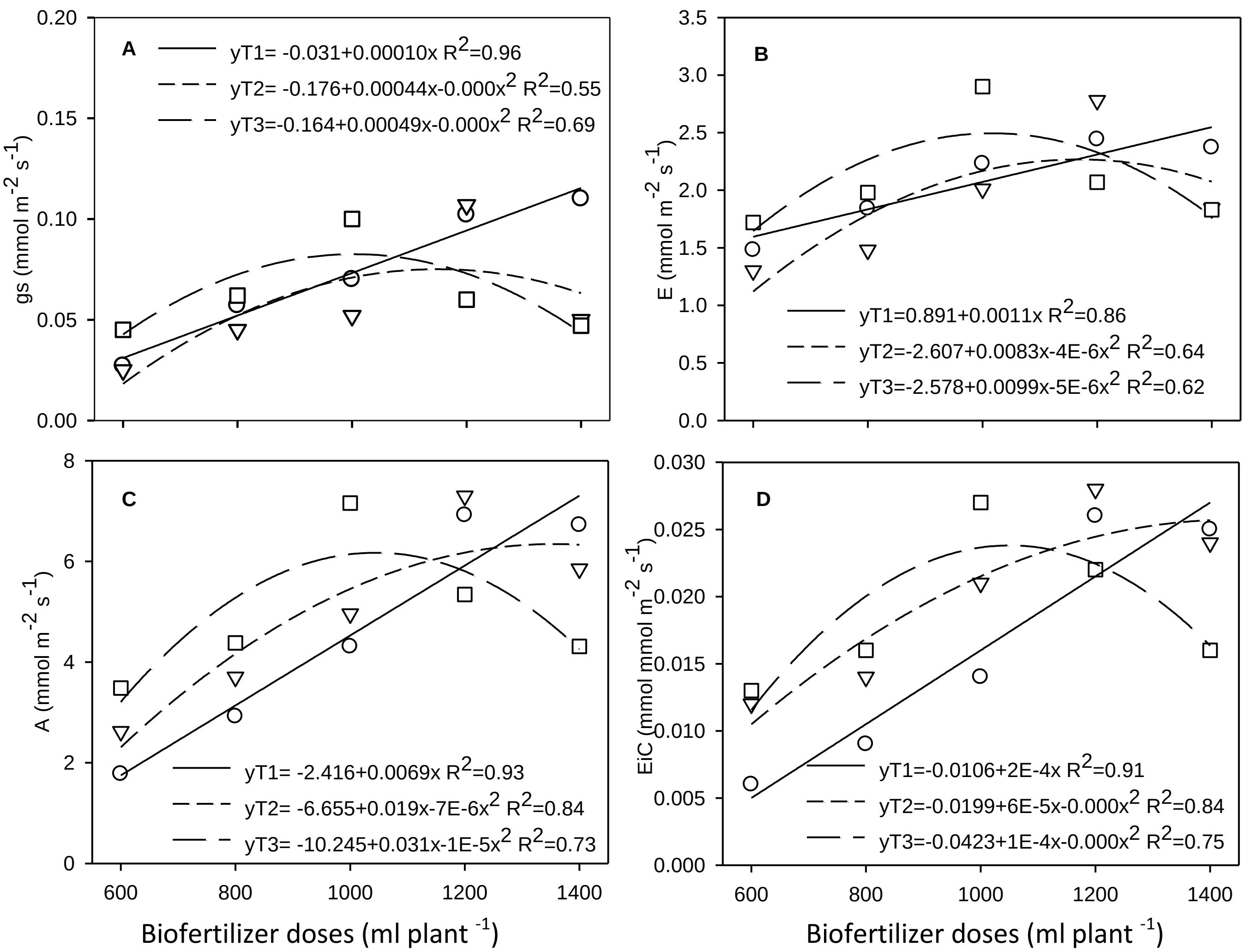

Figure 1. Stomatal conductance - gs (A), transpiration - E (B), photosynthetic rate - A (C) and instantaneous carboxylation efficiency - EiC (D) in tomato plants grown with different organic fertilizers and biofertilizer doses.

maximum of 1.70 and 2.32 mmol H_2O m^{-2} s^{-1} at the estimated doses of 1037.5 and 990 mL $plant^{-1}$, respectively (Figure 1B).

The photosynthetic rate also increased linearly in T1, reaching 320% with the addition of biofertilizer doses until 1400 ml $plant^{-1}$, while in T2 and T3 the photosynthesis reached the maximum of 6.23 and 6.25 µmol CO_2 m^{-2} s^{-1}, with the estimated doses of 1357 and 1049 ml $plant^{-1}$, respectively (Figure 1C). Likewise, EiC increased by approximately 380% with increases in biofertilizer doses in T1, while in T2 and T3 the maximum EiC of 0.023 and 0.017 µmol m^{-2} s^{-1} was reached in the estimated doses of 1250 and 1000 ml $plant^{-1}$, respectively (Figure 1D).

The stomatal conductance, transpiration, photosynthesis and carboxylation efficiency had the same behavior in each type of fertilizer, indicating that both water loss and carbon fixation were influenced by stomatal control, as observed in tomato under drought stress (Morales et al., 2015). In the T1 fertilizer, the linear growth of these characteristics with the increase of the biofertilizer doses indicates that the tomato plants cultivated with 20% earthworm humus use the incorporation of biofertilizer until 1400 mL plant^{-1}, unlike T2 and T3 that decreased the gas exchange of the tomato with the increase of the doses of biofertilizer. These results probably occurred due to the concentration of the types of fertilizers, because as in T1 the proportion of soil was higher, the increase of the doses of biofertilizer favored in the increase of the gas exchanges by supplying nutrients in a gradual way, complementing the hummus. It was observed in tomato plants under different organic fertilizers, that in the treatments with 25% earthworm humus, the plants had lower height and root length, compared to treatments composed of 50 and 100% humus (Oliveira et al., 2013a), demonstrating that the low amount of this organic fertilizer is insufficient in the nutrition of the tomato plants and, therefore the doses of biofertilizer were useful in the mineral supplementation of the substrate.

On the other hand, in the substrates with goat manure and cattle, the highest doses of biofertilizer were more than necessary for tomato plants, as observed by reductions of the gas exchanges at the highest applied dose. This could possibly be due to nutrient supply in addition to that the plants especially needed macronutrients, as occurred in okra plants in which cattle manure increased foliar N, P and K when associated to the application of the biofertilizer (Oliveira et al., 2014). Also, the increase of the doses of biofertilizer associated with the concentration of manure in both substrates may have compromised the growth of tomato plants. In the watermelon culture, it was observed that the increase in doses (L pit^{-1}) of both goat and cattle manure had a threshold for plant length and diameter (Cavalcante et al., 2010).

The lowest leaf temperature of 34.05 °C was reached with the maximum estimated dose of 1037.4 mL plant^{-1}, regardless of the type of fertilizer used (Figure 2A). However, among types, the internal CO_2 concentration was higher in T1, followed by T3 and T2 (Figure 2B).

The low stomatal conductance reduces transpiration, decreasing the cooling capacity of the leaf and increasing its temperature, as observed in plants of all types of fertilizer with the lowest dose of biofertilizer, and in manure treatments associated with a higher dose, which seems to have configured nutritional excess, since the characteristics of gas exchange are useful in the interpretation of physiological changes in the plants when subjected to adverse conditions, such as low and high amount of nutrients (Gondim et al., 2015). In other words, high gs leads to the increase of Ci (Santos et al., 2010), as observed in this study, in which the linear increase of stomatal conductance in T1 fertilizer led to higher internal carbon concentration. The WUE increased approximately 122% with the addition of biofertilizer doses, regardless of the type of fertilizer used (Figure 2C). Already, the SPAD chlorophyll content was higher in the T2 and T3 fertilizers (Figure 2D), while among the biofertilizer doses, the maximum SPAD was 42.6% reached at the maximum estimated dose of 999.5 mL plant^{-1} (Figure 2E).

The increase in the WUE in relationship with the increase in the doses of biofertilizer may be due to the lower transpiration in relation to the photosynthetic rate, or the higher nutrient supply by increasing the doses of biofertilizer (Oliveira et al., 2014), as verified in lettuce plants in which organic fertilization improved water use efficiency (Santos et al., 2010). It was also verified in tomato plants, that organic fertilizers contribute to the adequate growth and development of plants and to the correction of nutritional deficiencies (Dinu et al., 2015; Kalbani et al., 2016).

In a study with lettuce plants, was observed that the SPAD chlorophyll content was not altered by organic fertilizer types, among them the cattle manure (Santos et al., 2010) differing from the results of this work, in which the substrates with goat manure had higher SPAD. Furthermore, the higher dose of biofertilizer decreased the chlorophyll content of tomato plants, a fact that may have led to the reduction of gas exchange. The chlorophyll content was not altered in tomato plants fertilized adequately (Zhu et al., 2012), but reduced in wheat and rice plants with the increase of N supply, configuring excess of this nutrient (Swain and Sandip, 2010; Hasan et al., 2016). This fact probably occurred in this study in tomato plants fertilized with bovine and goat manure together with the highest doses of biofertilizer. The root-shoot ratio increased linearly 27.4% with the addition of biofertilizer doses in the T1 fertilizer, while in T2 and T3 this ratio was maximum of 0.213 and 0.205, respectively, in the estimated dose of 1000 mL plant^{-1} for both types of fertilizer (Figure 2F).

During plant growth, fertilizers present on the substrates, especially T2 and T3, coupled with the supply of the biofertilizer and nutrients contained in the soil, the nutritional requirements of the crop beyond the appropriate, as evidenced by the increase of the root-shoot ratio, besides the gas exchange and the chlorophyll content SPAD. The lower root-shoot ratio is a reflection of the reduction in the dry mass of the roots, as observed with the higher doses of biofertilizer and the fertilizers T2 and T3, which can occur due to the excess of nutrients released with the increase of the organic fertilization, as well as observed in okra plants grown on grape marc substrate and fertilized with slow-release fertilizers (GOMES et al., 2017), or can be attributed to increased electrical conductivity with organic fertilizers (Ersahin et al., 2017).

On the other hand, the reduction of root-shoot ratio

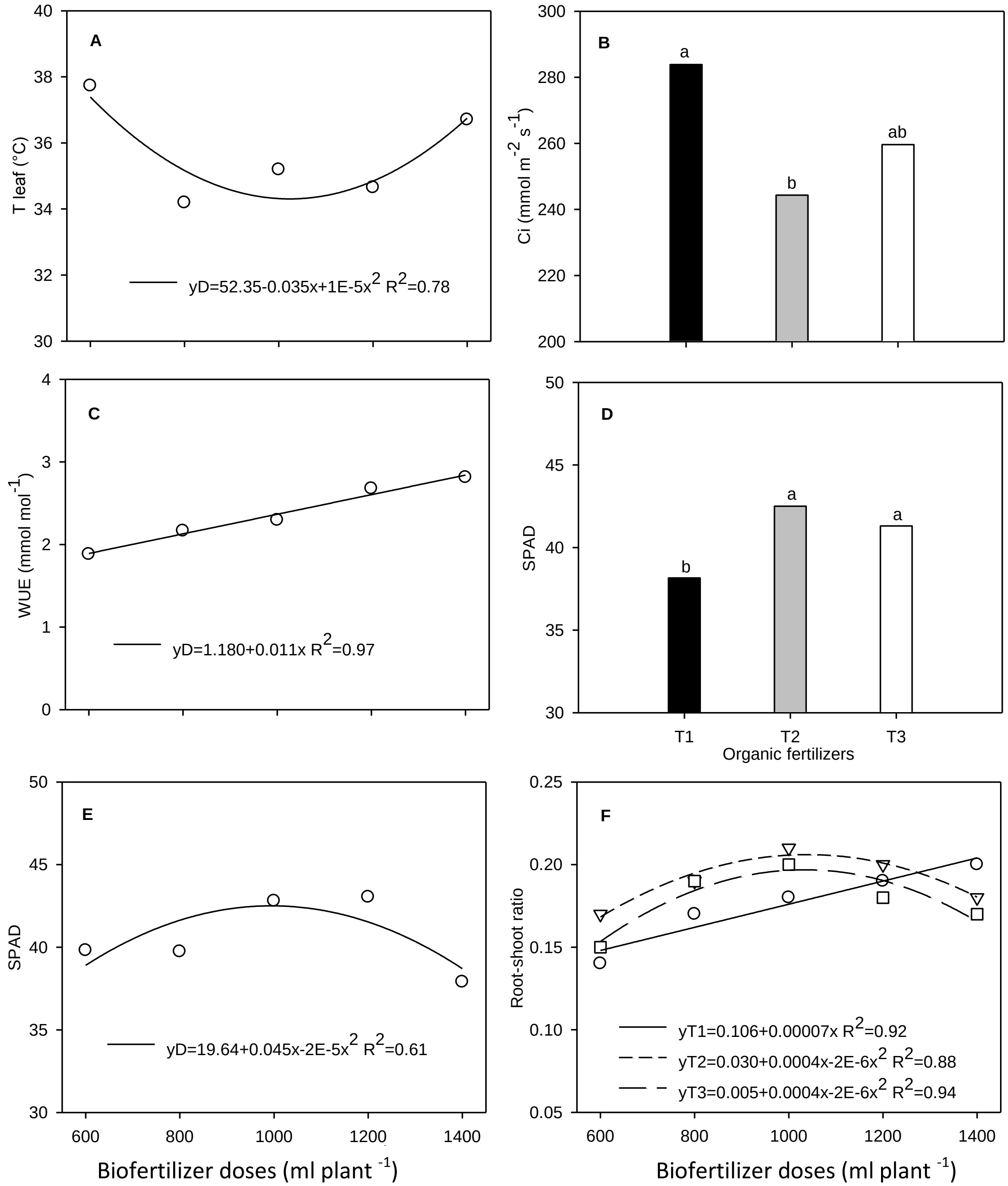

Figure 2. Leaf temperature - Tleaf (A), internal CO_2 concentration - Ci (B), instantaneous water use efficiency - WUE (C), chlorophyll content SPAD (D and E) and root-shoot ratio (F) in tomato plants grown with different organic fertilizers and biofertilizer doses.

observed in plants cultivated on manure substrates and high doses of biofertilizer may mean that these plants, once the needs of the root growth have been satisfied by the greater absorption of the nutrients provided by the biofertilization, have passed; they direct their activities to the formation of the aerial part, such as leaves and fruits (Oliveira et al., 2014; Cruz et al., 2015; Kalbani et al., 2016).

Conclusion

The concentration of organic fertilizer in the soil and the sociation with doses of biofertilizer influence the gas exchange and SPAD chlorophyll content in tomato plants. In addition, increasing doses of biofertilizer with organic fertilizer (T1) increase the gas exchange in tomato plants, while high doses together with more concentrated organic fertilizers (T2 and T3), decreases these characteristics.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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