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Trocas gasosas foliares e índices de clorofila em goiabeira fertirrigada com biofertilizante bovino e nitrogênio

Leaf gas exchange and chlorophyll index in guava fertirrigated with bovine biofertilizer and nitrogen

Intercambio de gases foliar e índice de clorofila en guayaba fertilizada con biofertilizante bovino y nitrógeno

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Resumo

O objetivo deste trabalho foi avaliar a influência da fertirrigação com nitrogênio e biofertilizante líquido bovino nas trocas gasosas e índices de clorofila foliar da goiabeira 'paluma' (*Psidium guajava* L.). O delineamento experimental foi em blocos casualizados com tratamentos distribuídos em esquema fatorial (2 × 4) referente à fertilização mineral com N (50% e 100% do N recomendado) e concentrações de biofertilizante (0, 2,5, 5,0 e 7,5% do volume fertirrigado). As variáveis avaliadas foram índices de clorofila *a* (Chl *a*), clorofila *b* (Chl *b*) e clorofila total (Chl_{total}), concentração interna de CO_2 (*Ci*), condutância estomática (gs), taxa transpiratória (E), fotossíntese líquida (*A*), eficiência instantânea da carboxilação (*EiC*) e eficiência no uso de água (*EUA*). O biofertilizante afetou significativamente Chl *a*, Chl *b*, Chl_{total}, *A*, *gs* e *E*, com ajuste polinomial quadrático dos resultados. Porém, não houve efeito da fertilização com N e da interação entre os fatores. O máximo índice de Chl_{total} foi 32,31 obtido com a dose estimada de 3,8% do biofertilizante, enquanto *A*, *gs* e *E*, obtiveram máximas respostas de 19,09 µmol do CO_2 m^{-2} s^{-1}, 0,28 mol m^{-2} s^{-1} e 4,93 mmol de H_2O m^2 s^{-1}, com as doses estimadas de 3,6%, 3,6% e 3,7%, respectivamente. Em geral, o biofertilizante bovino líquido aplicado via fertirrigação afeta positivamente as respostas fotossintéticas em goiabeira ‘paluma’, porém, com efeitos decrescentes para doses superiores a 3,8%.

Palavras-chave: *Psidium guajava* L.; Fotossíntese líquida; Nutrição de plantas.

Abstract

The objective of this work was to evaluate the influence of fertirrigation with nitrogen and liquid bovine biofertilizer on gas exchange and leaf chlorophyll index of 'paluma' guava (*Psidium guajava* L.). The experimental design was randomized blocks with treatments distributed in a factorial arrangement (2 × 4) referring to mineral fertilizing with N (50% and 100% of N recommended) and biofertilizer concentrations (0, 2.5, 5.0 and 7.5% of the fertirrigated volume). Variables evaluated were chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll indexes (Chl_{total}), internal CO_2 concentration (*Ci*), stomatal conductance (*gs*), transpiration (*E*), net photosynthesis (*A*), instant carboxylation efficiency (*iCE*) and water use efficiency (*WUE*). The biofertilizer significantly affected Chl *a*, Chl *b*, Chl_{total}, *A*, *gs* and *E*, with quadratic polynomial adjustment of the results. However, there was no effect of N fertilization and interaction between the factors. Maximum index of Chl_{total} was 32.31 obtained with the estimated dose of 3.8% of the biofertilizer; while *A*, *gs* and *E* presented maximum responses of 19.09 µmol of CO_2 m^{-2} s^{-1}, 0.28 mol of H_2O m^{-2} s^{-1} and 4.93 mmol of H_2O m^{-2} s^{-1}, with estimated doses of 3.6%, 3.6%, and 3.7%, respectively. Generally, liquid bovine biofertilizer applied via fertirrigation affects positively the photosynthetic responses in 'paluma' guava, however, with decreasing effects for doses above 3.8%.

Keywords: *Psidium guajava* L.; Net photosynthesis; Plant nutrition.

Resumen

El objetivo de este trabajo fue evaluar la influencia de la fertirrigación con nitrógeno y el biofertilizante líquido bovino en el intercambio de gases y el índice de clorofila foliar de la guayaba 'paluma' (*Psidium guajava* L.). El diseño experimental fue en bloques al azar con tratamientos distribuidos en esquema factorial (2 × 4) referidos a fertilización mineral con N (50% y 100% del N recomendado) y concentraciones de biofertilizante (0; 2,5; 5,0 y 7,5 % del volumen fertilizado). Las variables evaluadas fueron clorofila *a* (Chl *a*), clorofila *b* (Chl *b*), clorofila total (Chl_{total}), concentración interna de CO_2 (*Ci*), conductancia estomática (*gs*), tasa de transpiración (*E*), fotosíntesis líquida (*A*), eficiencia de carboxilación instantánea (*ECi*) y eficiencia en el uso del agua (*EUA*). El biofertilizante afectó significativamente a Chl *a*, Chl *b*, Chl_{total}, *A*, *gs* y *E*, con ajuste polinomial cuadrático de los resultados. Sin embargo, no hubo efecto de la fertilización con N y de la interacción entre factores. El índice Chl_{total} máximo fue 32,31 obtenido con la dosis estimada de 3,8% del biofertilizante, mientras que *A*, *gs* y *E*, obtuvieron respuestas máximas de 19,09 µmol de CO_2 m^{-2} s^{-1}, 0,28 mol m^{-2} s^{-1} y 4,93 mmol de H_2O m^2s^{-1}, con dosis estimadas de 3,6%, 3,6% y 3,7%, respectivamente. En general, el biofertilizante líquido bovino aplicado vía fertirrigación afecta positivamente las respuestas fotosintéticas en guayaba 'paluma', sin embargo, con efectos decrecientes para dosis superiores al 3,8%.

Palabras clave: *Psidium guajava* L.; Fotosíntesis líquida; Nutrición vegetal.

1. Introduction

Guava (*Psidium guajava* L.) is a fruit of the Myrtaceae family, originally from South and Central America, and widely cultivated in Brazil, mainly in its semiarid portion. In 2018, the national production of guava was 578,608 Mg (IBGE, 2019), being cv. Paluma the most cultivated by producers and the best accepted by the consumer market.

Despite being considered a plant tolerant to low fertility soils, to obtain high yields the nutritional needs of guava must be adequately provided, and the correct management of fertilization is essential (Corrêa et al., 2018). In this sense, besides supplying with inorganic sources of nutrients, a complementary fertilizing with biofertilizer presents promising results in the production and quality of guava fruits (Santana et al., 2017).

Biofertilizer's benefits are associated not only by its ability to supply nutrients directly but also, because it is rich in microorganisms capable of converting nutritionally important elements into forms available through biological processes (Cavalcante et al., 2019; Yadav & Sarkar, 2019). In tamarind (*Tamarindus indica* L.) the use of biofertilizer promoted an increase in photosynthetic pigments and the attenuation of the negative effects of salinity on the growth of seedlings (Lima Neto, 2018). In passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Deg.), there was an increase in the internal concentration of CO_2 by reducing stomatal conductance and transpiration, however, without altering the net assimilation of carbon dioxide (Freire et al., 2014). In melon (*Cucumis melo* L.) the use of bovine biofertilizer provided an increase of photosynthetic rates and transpiration, in addition to an adequate supply of K and N (Viana et al., 2013).

N is the second most demanded nutrient for guava tree (Natale et al., 2009) and the influence of nitrogen fertilization on the physiological characteristics of guava has been recently studied. Bezerra et al. (2018) evaluated the effects of nitrogen on the gas exchange of guava cv. Paluma grown in saline soil; while Silva et al. (2017) studied the effects of salinity of irrigation water on the photosynthetic pigment contents and leaf morphophysiology of guava seedlings cv. Paluma under nitrogen fertilization. However, the combined effects of nitrogen fertilization with bovine biofertilizer on the photosynthetic characteristics of guava cv. Paluma are unknown.

Therefore, our research aimed to evaluate the levels of leaf chlorophyll and gas exchange of guava cv. Paluma fertirrigated with liquid bovine biofertilizer and nitrogen in tropical semiarid conditions.

2. Material and Methods

The experiment was carried out from July 2014 to August 2015 at the experimental farm of the Federal University of São Francisco Valley, Petrolina City, Pernambuco State, Brazil (9°19'10.9"S 40°33'48.1"W). The local climate is classified as BSh that corresponds to a semiarid region (Alvares et al., 2013). During the execution of the experiment, the climatic data were monitored by a meteorological station installed in the experimental area, registering air temperature between 24 and 28ºC, relative humidity between 48 and 64% and accumulated precipitation of 650 mm.

The soil in the experimental area was classified as Yellow Argisol (Ultisol - American Classification of soil taxonomy), and before the installation of the experiment, the physical and chemical characterization of the soil was performed at depths 0-20 and 20-40cm (Table 1).

Table 1. Chemical and physical characteristics of the soil (0-20 and 20-40 cm depths) of the experimental area before the installation of the experiment.

Characteristics of the soil	Values	
	0-20 cm	20-40 cm
pH (measured in water)	6.2	5.4
Ca^{2+} ($cmol_c$ dm³)	2.1	2.0
Mg^{2+} ($cmol_c$ dm³)	1.4	1.2
Al^{3+} ($cmol_c$ dm³)	0.0	0.0
K^+ ($cmol_c$ dm³)*	0.74	0.63
Na^+ ($cmol_c$ dm³)	0.11	0.19
P (mg/dm³)*	207.0	58.0
O.M.(%)**	0.56	0.53
Clay (g kg^{-1})	72.0	83.0
Silt (g kg^{-1})	55.0	52.0
Sand (g kg^{-1})	873.0	865.0

*P, K: Mehlich-1; Ca, Mg: KCl 1M extractor; **O.M. (Organic Matter): muffle method 500ºC. Source: Elaborated by the authors (2020).

One-year-old guava trees cv. Paluma were used in this study. The plants were distributed in a 4 × 4 m spacing, irrigated daily by a micro-sprinkler system, with one emitter per plant with a flow of 42 L h^{-1}, and irrigation depths were calculated based on the daily evapotranspiration records and corrected according to the culture coefficient (Kc) corresponding to the phenological phases (Natale et al., 2009).

The experiment was carried out in randomized block design with treatments distributed in a factorial arrangement (2 × 4) referring to mineral fertilizing with N [fertilization with 50% and 100% of N recommended by Natale (2009)] and biofertilizer concentrations (0, 2.5, 5.0 and 7.5% of the fertirrigated volume), with four replications of five plants each.

The biofertilizer used in the experiment was composed of fresh bovine manure diluted in an equal volume of non-chlorinated water, and submitted to an anaerobic fermentation process for 30 days in an airtight container, as stated by Santos (1991). The application was carried out via irrigation water according to the doses corresponding to each treatment, with a frequency of each 15 days. The biofertilizer presented in its composition 0.72 g dm^{-3} of N, 0.04 g dm^{-3} of P, 0.50 g dm^{-3} of K, 0.20 g dm^{-3} of Ca, 0.12 g dm^{-3} of Mg and 0.39 g dm^{-3} of S, 4.0 mg dm^{-3} of B, 6.0 mg dm^{-3} of Cu, 77 mg dm^{-3} of Fe, 10 mg dm^{-3} of Mn, 16 mg dm^{-3} of Zn and 81 mg dm^{-3} of Na.

The nutrient management was carried through a fertigation system (Viqua® venture injector of 1” at 10 bar operating pressure), according to soil analysis (Table 1) biweekly, starting after production pruning until 20 days before harvest, using a formulated fertilizer composed by 12% of N, 5% of P, 11% of K, 13.1% of Ca and 0.2% of B. Treatments fertilized with 100% of N also received urea (45% of N). Zinc (Coda Zinc®, 10.4% of Zn), magnesium (Coda Mg®, 6.6% de Mg) and iron (Codamin Br®, 2.0% of Fe) were leaf applied. All cultural practices, such as pruning, weeds, pests, and diseases control were carried out following the instructions of Natale et al. (2009).

Chlorophyll indexes and gas exchange evaluations were performed after the physiological fall of the fruits (Pessarakli, 2002), in the morning, between 9:00 am and 11:00 am, on four leaves per plant, fully expanded and healthy at the median height of the canopy; for that, a chlorophyll meter was used (Falker®, Brazil) (El-Hendawy et al., 2005) and an infrared gas analyzer (IRGA), model LCi Portable Photosynthesis System® (ADC BioScientific Limited, UK), with temperature control at 25°C, irradiation of 1800 µmol photons m^{-2} s^{-1} and airflow of 200 ml min^{-1}.

At that time, chlorophyll *a*, chlorophyll *b* and total chlorophyll indexes (*a* + *b*) (ICF), internal CO_2 concentration (Ci - mmol of CO_2 m^{-2} s^{-1}), stomatal conductance (gs - mol of H_2O m^{-2} s^{-1}), transpiration (E - mmol of H_2O m^{-2} s^{-1}) and net photosynthesis (A - expressed in µmol of CO_2 m^{-2} s^{-1}) were measured. Additionally, the instant carboxylation efficiency ($iCE = A / Ci$) expressed in (µmol m^{-2} s^{-1}) / (mmol m^{-2} s^{-1}) and the water use efficiency ($WUE = A / E$) in (µmol of CO_2 m^{-2} s^{-1}) / (mmol of H_2O m^{-2} s^{-1}) were calculated.

The data were submitted to the residue normality test (Shapiro Wilk). Subsequently, the analysis of variance (ANOVA) was performed by the ‘F’ test ($P \leq 0.05$), and, based on the significance, the treatments with N were compared with each other and the concentrations of biofertilizer subjected to polynomial regression analysis. Statistical analysis was performed using the SISVAR software version 5.6 (Ferreira, 2011).

3. Results and Discussion

From the results of the analysis of variance, it was observed that the biofertilizer significantly influenced the chlorophyll *a* (Chl *a*), *b* (Chl *b*) and total (Chl_{total}), net photosynthesis (*A*), stomatal conductance (*gs*) and transpiration (*E*) (Table 2). However, there was no effect of nitrogen doses and the interaction between the factors studied on any of the variables.

Table 2. Analysis of variance by the F test for the indexes of chlorophyll *a* (Chl *a* - ICF), chlorophyll *b* (Chl *b* - ICF), total chlorophyll (Chl_{total} - ICF), internal CO_2 concentration (*Ci* - mmol of CO_2 m^{-2} s^{-1}), stomatal conductance (*gs* - mol of H_2O m^{-2} s^{-1}), transpiration (*E* - mmol of H_2O m^{-2} s^{-1}), net photosynthesis (*A* - µmol of CO_2 m^{-2} s^{-1}), instant carboxylation efficiency [*iCE* - (µmol m^{-2} s^{-1}) / (mmol m^{-2} s^{-1})] and the water use efficiency [*WUE* - (µmol of CO_2 m^{-2} s^{-1}) / (mmol of H_2O m^{-2} s^{-1})] of guava cv. Paluma fertirrigated with liquid bovine biofertilizer and nitrogen.

Source of variation	Chl *a*	Chl *b*	Chl_{total}	*A*	*Ci*	*gs*	*E*	*iCE*	*WUE*
Biofertilizer (B)	5.41^{**}	6.35^{**}	6.28^{**}	3.06^{*}	1.39^{ns}	2.92^{*}	2.97^{*}	2.10^{ns}	1.22^{ns}
Nitrogen (N)	0.006^{ns}	0.002^{ns}	0.002^{ns}	0.05^{ns}	0.026^{ns}	0.009^{ns}	0.04^{ns}	0.08^{ns}	0.02^{ns}
50%	25.67	4.59	30.26	17.23	236.56	0.24	4.36	0.07	4.03
100%	25.63	4.60	30.23	17.03	235.27	0.24	4.30	0.07	4.05
MSD	1.18	0.42	1.52	1.86	16.58	0.04	0.56	0.02	0.32
B x N	0.072^{ns}	1.08^{ns}	0.23^{ns}	2.39^{ns}	0.57^{ns}	1.97^{ns}	1.86^{ns}	2.02^{ns}	0.05^{ns}
CV (%)	6.31	12.43	6.85	14.83	9.56	23.3	17.69	12.82	10.96

**significant at P <0.01; *significant at P <0.05; ns: not significant. Means followed by the same letter in the columns are similar to each other. MSD: Minimum significant difference, CV: Coefficient of variation. Source: Elaborated by the authors (2020).

The responses obtained for Chl *a*, Chl *b*, Chl_{total}, *A*, *gs* and *E* of guava cv. Paluma as a function of the bovine biofertilizer presented a quadratic polynomial adjustment (Figure 1). Chl *a* index obtained a maximum response of 27.26 ICF with the estimated dose of 3.7% of the biofertilizer (Figure 1A), while for Chl *b* index, the maximum response and estimated dose were 5.1 ICF and 4.0%, respectively (Figure 1B). Maximum Chl_{total} index was 32.31 ICF obtained with the 3.8% dose (Figure 1C).

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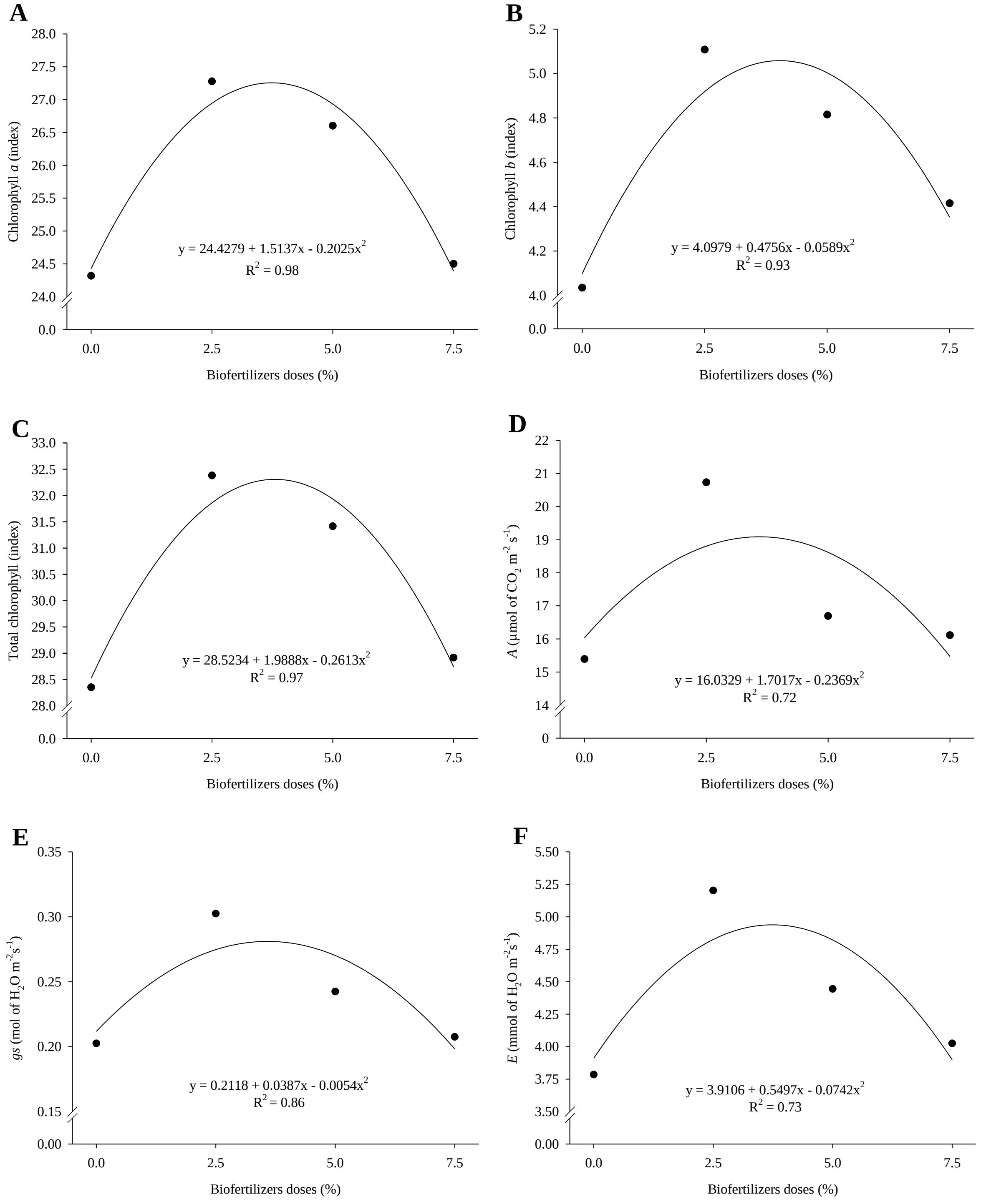
Figure 1. Indexes of chlorophyll *a* (Chl *a*) (A), chlorophyll *b* (Chl *b*) (B) and total chlorophyll (Chl_{total}) (C), net photosynthesis (*A*) (D), stomatal conductance (*gs*) (E) and transpiration (*E*) (F) of guava cv. Paluma fertirrigated with liquid bovine biofertilizer.



Source: Elaborated by the authors (2020).

The increase provided for Chl *a*, Chl *b* and Chl_{total} indexes by the biofertilizer compared with the control treatment (0.0% of the biofertilizer) is due, indirectly, to the biofertilizer's ability to improve the physical, chemical and biological properties of the soil (Wang et al., 2019), increasing the availability of soil nutrients for plants (Yadav & Sarkar, 2019), favoring a better nutritional status. The increase in chlorophyll indexes provided by the biofertilizer is beneficial for guava because the conversion of absorbed solar radiation into stored chemical energy occurs in the chlorophyll molecules, through the capture of light energy and the transfer of excitation energy to the centers of reaction (Alton, 2017).

However, it is known that the effects of biofertilizer vary depending on the dose (Aguiar et al., 2017) and that its benefits are limited, which was clearly demonstrated in our study because after reaching the maximum chlorophyll levels with an approximate dose of 4%, the plants presented an accentuated decrease. The increase in the synthesis of photosynthetic pigments promoted by the biofertilizer is more specifically related to the presence of nitrogen (0.72 g dm^{-3}) and magnesium (0.12 g dm^{-3}), which are structural elements of chlorophylls and carotenoids (Taiz et al., 2017), these two nutrients plus potassium are the components present in higher concentrations in the bovine biofertilizer used.

Taking into account the diversity of nutritional components of bovine biofertilizer, the reduction in responses to chlorophyll indexes promoted by high doses of biofertilizer may be associated with the nutritional imbalance caused by the excess of its components (Viana, 2013), as the demand for nutrients differs between species, cultivars and development stages of the same plant, the effects of biofertilizer also vary depending on these factors and can be harmful when in excess.

Another condition that may have favored the reduction of chlorophyll indexes in plants treated with doses higher than 4% was the sodium content present in bovine biofertilizer (81 mg dm^{-3}), according to Munns and Tester (2008) when there is an excess of salts in leaf tissues, the activity of the enzyme chlorophyllase, responsible for the degradation of chlorophyll and chloroplasts, is stimulated promoting loss of photosynthetic activity of pigmentation proteins. In melon (*Cucumis melo* L.), biofertilizers with high levels of sodium reduced the physiological responses of plants (Viana et al., 2013). Although, in guava cv. Paluma symptoms of phytotoxicity have not been observed, the reduction in chlorophyll contents is an indicative of the harmful effects caused by high doses of the biofertilizer.

In addition to the factors already mentioned, chlorophyll indexes are influenced proportionally by the levels of leaf nitrogen (Schlemmer et al., 2013), therefore, as no differences were found between the chlorophyll indexes of the plants that received the different of N fertilization, it is understood that using the lowest dose (50%) was sufficient to obtain satisfactory levels of the nutrient in the plant, because when nitrogen is in excess, responses to chlorophyll levels are not affected.

Net photosynthesis obtained the maximum response of 19.09 µmol of CO_2 m^{-2} s^{-1}, with the estimated dose of 3.6% of the biofertilizer. The maximum estimated net photosynthesis obtained in this study is higher than that recorded by Nava et al. (2009), corresponding to 16 µmol of CO_2 m^{-2} s^{-1} in guava trees cv. Media China evaluated at the same time of day and under conditions of ideal water supply, thus demonstrating the benefit of using the biofertilizer in adequate doses. Similar to photosynthesis, stomatal conductance reached its maximum response with the estimated dose of 3.6% of the biofertilizer, corresponding to 0.28 mol of H_2O m^{-2} s^{-1}. Guava trees cv. Paluma of all treatments in this study were under the same water supply condition, therefore, the reduction in stomatal conductance observed with the use of biofertilizer doses above 3.6% is possibly associated with the osmotic and ionic effects of high sodium content in higher dosages, which compromises the water absorption by the plant, while in the lower doses, the beneficial effects of the biofertilizer prevailed. Bezerra et al. (2018) when studying the gas exchange of guava cv. Paluma grown in salinized soils, observed a reduction in gs from 0.20 to 0.12 mol of H_2O m^{-2} s^{-1} with the increase in the electrical conductivity of the soil, both values are lower than those registered in our study with the doses of biofertilizer, for the authors, the variation is due to the guava water regulation mechanism when exposed to variation in soil water potential.

Additionally, stomatal conductance regulates the diffusion of CO_2 and H_2O, as both share a common path through the stomatal pore; therefore, the similarity observed between stomatal conductance and transpiration responses as a function of biofertilizer doses (Figures 1E e 1F) reinforce the existence of this relation. The maximum transpiration of 4.93 mmol of H_2O m^{-2} s^{-1} was estimated for the 3.7 dose of biofertilizer (Figure 1F). However, the internal CO_2 concentration (Table 2) did not vary as a function of the treatments with biofertilizer, presenting an average value of 235.91 µmol mol^{-1}.

Nava et al. (2009), observed in guava cv. Media China the existence of an opposite relation between the internal concentration of CO_2 and the photosynthetic rate, because with the reduction of photosynthesis, CO_2 is less required by the photosynthetic process, accumulating in the intercellular space. In our study, however, CO_2 did not vary due to the oscillation in net photosynthesis. When CO_2 is not a limiting factor, photosynthesis tends to be approximately proportional to the density of available light (Lopes & Lima, 2015), thus the increase in net photosynthesis up to the 3.6 dose (Figure 1D) caused by the biofertilizer is probably associated with an increase in the total chlorophyll index up to the 3.8 dose (Figure 1C), that provided better light absorption.

Despite the differences in net photosynthesis, the instantaneous carboxylation efficiency and water use efficiency variables were not affected by the biofertilizer doses, with their average values being 0.07 (μmol m^{-2} s^{-1}) / (mmol m^{-2} s^{-1}) and 4.04 (μmol of CO_2 m^{-2} s^{-1}) / (mmol of H_2O m^{-2} s^{-1}), respectively.

4. Conclusion

Nitrogen alone or combined with liquid bovine biofertilizer did not present a synergistic action on the evaluated characteristics of ‘Paluma’ guava. The bovine liquid biofertilizer influenced the levels of leaf chlorophyll and gas exchange of the 'Paluma' guava, up to an approximate dose of 3.8%. Further work should be carried out by evaluating other nitrogen doses combined with liquid bovine biofertilizer.

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