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Gas exchange and photosynthetic pigments in zucchini plants under cattle bio-fertilization and sources of nitrogen

Trocas gasosas e pigmentos fotossintéticos em abobrinha adubada com biofertilizante bovino e fontes de nitrogênio

Intercambios de gas y pigmentos fotosintéticos en calabinas fertilizados con biofertilizante bovino y fuentes de nitrógeno

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Abstract

Zucchini is traditionally used in human consumption, however, the scarcity of information, especially about fertilization and the physiological aspects of this crop, constitutes a major obstacle its cultivation and commercialization. The objective was evaluate the chlorophyll content and gas exchange under cattle bio-fertilization and different sources of nitrogen. The work was carried a randomized block experimental design was used in a factorial scheme 5 x 3 + 1 composed of five cattle bio-fertilizer concentrations, three sources of nitrogen and an additional treatment without nitrogen. The levels of chlorophyll *a*, *b* and total, liquid photosynthesis, transpiration, internal CO_2 concentration, stomatal conductance, instantaneous water use efficiency, and instantaneous carboxylation efficiency were evaluated. The studied variables were influenced by the interaction between the concentrations of the cattle bio-fertilizer and the nitrogen sources, except for the chlorophyll *b* content and the instantaneous water use efficiency, which responded only to the nitrogen sources. Ammonium sulfate and urea increased increase the physiological variables evaluated. The combination of ammonium sulfate with bio-fertilizer promoted an increase in chlorophyll *a*, total chlorophyll, liquid photosynthesis, transpiration, internal carbon concentration, stomatal conductance, and instantaneous carboxylation efficiency. The cattle bio-fertilizer in the soil without nitrogen did not increase the physiological variables evaluated.

Keywords: *Cucurbita pepo* L; Physiology; Chlorophyll contents; Organic fertilization; Nitrogen fertilization.

Resumo

A abobrinha é utilizada tradicionalmente na alimentação da população, no entanto, a escassez de informações, sobretudo para a adubação e os aspectos fisiológicos da cultura, constitui-se em grande entrave para melhoria no cultivo e comercialização. Objetivou-se avaliar os teores de clorofilas e trocas gasosas em abobrinha adubada com biofertilizante bovino e diferentes fontes de nitrogênio. O trabalho foi realizado em delineamento experimental de blocos casualizados em esquema fatorial 5 x 3 + 1. Sendo, cinco concentrações de biofertilizante bovino, três fontes de nitrogênio e um tratamento adicional sem nitrogênio. As variáveis analisadas foram teores de clorofila *a*, *b* e total, fotossíntese líquida, transpiração, concentração interna de CO_2, condutância estomática, eficiência instantânea no uso da água e eficiência instantânea de carboxilação. Pelos resultados, as variáveis estudadas foram influenciadas pela interação entre as concentrações de biofertilizante bovino e as fontes de nitrogênio, com exceção do teor de clorofila *b* e da eficiência instantânea no uso da água que

responderam apenas às fontes de nitrogênio. O sulfato de amônio e a ureia estimularam a formação de clorofila *b* e a eficiência instantânea no uso da água. A combinação do sulfato de amônio com biofertilizante proporcionou aumentos na clorofila *a*, clorofila total, fotossíntese líquida, transpiração, concentração interna de carbono, condutância estomática e eficiência instantânea de carboxilação. O biofertilizante bovino no solo sem nitrogênio não proporcionou aumento nas variáveis fisiológicas avaliadas.
Palavras-chave: *Cucurbita pepo* L; Fisiologia; Teores de clorofila; Adubação orgânica; Adubação nitrogenada.

Resumen

El calabacín se utiliza tradicionalmente para alimentar a la población, sin embargo, la escasez de información, principalmente para la fertilización y los aspectos fisiológicos del cultivo, constituye un gran obstáculo para mejorar el cultivo y la comercialización. El objetivo fue evaluar el contenido de clorofilas y el intercambio de gases en calabacines fertilizados con biofertilizante bovino y diferentes fuentes de nitrógeno. El trabajo se realizó en un diseño de bloques al azar en un esquema factorial 5 x 3 + 1, con cinco prácticas de biofertilizante bovino, tres fuentes de nitrógeno y un tratamiento adicional sin nitrógeno. Las variables analizadas fueron niveles de clorofila a, by total, fotosíntesis líquida, transpiración, concentración interna de CO2, conductancia estomática, eficiencia de uso instantáneo de agua y eficiencia de carboxilación instantánea. A partir de los resultados, las variables estudiadas fueron influenciadas por la interacción entre las fuentes de biofertilizante bovino y las fuentes de nitrógeno, con excepción del contenido de clorofila b y la eficiencia instantánea en el uso del agua que respondía solo a las fuentes de nitrógeno. El sulfato de amonio y la urea estimularon la formación de clorofila by la eficiencia instantánea en el uso del agua. Una combinación de sulfato de amonio con biofertilizante proporcionó incrementos en la clorofila a, clorofila total, fotosíntesis líquida, transpiración, concentración de carbono interno, conductancia estomática y eficiencia de carboxilación instantánea. El biofertilizante bovino en suelo sin nitrógeno no incrementó las variables fisiológicas evaluadas.
Palabras clave: Cucurbita pepo L; Fisiología; Contenido de clorofila; Fertilización orgánica; Fertilización con nitrógeno.

1. Introduction

Zucchini (*Cucurbita pepo* L.), in Brazil, it stands among the top ten vegetables of greatest production and economic value (Kumar et al., 2016), mainly in the Center and South regions of the country, and is traditionally used for human consumption, especially in the northeast.

According to Purquerio et al. (2019) the productivity of the crop is highly variable depending on the technology level and nutritional recommendation used. The scarcity of information, especially about fertilization (organic and nitrogenous) and the physiological aspects of this crop, is a major obstacle to the rational exploration that due to the lack of adequate knowledge has caused productivity loss.

The use of organic matter in combination with mineral fertilization increases the absorption of macro and micronutrients by plants and improves the physical, chemical, and biological characteristics of the soil, which results in increased plant growth and development. Among the sources of organic matter to be used, the cattle bio-fertilizer, the final residue of the fermentation of organic compounds that contain living or latent cells of microorganisms, when applied to the soil contributes to a balanced supply of nutrients (Silva et al., 2019).

Nitrogen, the second most required nutrient for vegetables, is an essential nutrient, determinant for plant production and development, is found in the soil in organic and inorganic forms, being absorbed by plants mainly in the form of nitrate (NO_3^-) and ammonium (NH_4^+). It is the constituent of many organic compounds, nucleic acids, and proteins, indispensable for the synthesis of chlorophyll and, therefore, in the photosynthesis process (Taiz et al., 2015).

Nitrogen fertilization needs to be performed in higher quantities and more frequently, considering the crop requirements, climate conditions, plant age, and the use efficiency of nitrogen sources, which is mostly unknown (Silva et al., 2015). Approximately 90% of the biological production of plants occurs in response to photosynthetic activity. The increase in stomatal resistance can cause a decrease in liquid photosynthesis. Thus, the verifying gas exchange is an important tool to determine plants as an alternative for cultivation, since growth decrease (and the consequent decrease in productivity) may be related to the reduction in photosynthetic activity (Taiz et al., 2015).

This study aimed to evaluate the chlorophylls content levels and gas exchange in zucchini plants under cattle bio-fertilization and different nitrogen sources.

2. Methodology

This study was carried in field experiment in Areia, Paraiba, Brazil (6° 57' 46" S 35° 41' 31" O, altitude of 623 m). The average annual temperature ranges from 23 to 24ºC and the relative air humidity is close to 84%. The soil of the experimental area was classified as Neolithic Regolitic, Psamitic typical, sandy loam texture. The climatic data for the experimental period were obtained at Meteorological Station A 310 the average rainfall was 167.22 mm, average air temperature was 21.07 ºC and the relative humidity was 88.5 %

A randomized blocks experimental design was used, with treatments distributed in a factorial scheme 5 x 3 + 1, represented by five concentrations of cattle bio-fertilizer (0, 10, 20, 30 and 40%) combined factorially with three nitrogen sources: nitrate of calcium, ammonium sulfate, and urea, and an additional treatment without nitrogen, in three replications. Each plot consisted of 20 plants distributed in four rows with five plants spaced 1.2 m x 0.6 m. Six plants from the two central rows were analyzed (Pôrto et al., 2014).

Five seeds of the zucchini cultivar Caserta were sown, and 15 days after sowing (DAS), when the plants had two definitive leaves a thinning was performed leaving one plant per pit. For the fertilization, 120 kg ha^{-1} of N (sources described in the experimental design), 40 kg ha^{-1} of P_2O_5 (triple superphosphate), 180 kg ha^{-1} of K_2O (potassium chloride) and 15 t ha^{-1} of cattle manure were applied. Phosphorus and cattle manure were distributed in the pits one week before sowing, along with 30 and 40% of nitrogen and potassium, respectively. The remaining nitrogen and potassium were applied in equal parts at the thinning and at the flowering stage at 45 DAS.

The chemical attributes of the bio-fertilizer were: N = 0.45 g L^{-1}; P = 0.22 g L^{-1}; K^+ = 0.27 g L^{-1}; Ca^{+2} = 0.21 g L^{-1}; Mg^{+2} = 0.13 g L^{-1}; organic matter = 19.02 g L^{-1}. The bio-fertilizer was applied at 15, 30, 45 and 60 DAS, in a volume of 500 mL per plant of each concentration described above. In treatments without bio-fertilizer, water was applied in a volume equivalent to that of the organic input. The bio-fertilizers were prepared according to Santos (1992) and consisted of anaerobic fermentation for 30 days in a plastic container, in a mixture containing fresh cattle manure and water in a proportion of 50% (v/v). To obtain the anaerobic system, the mixture was placed in a 240-liter plastic drum, leaving a space of 15 to 20 cm inside, hermetically closed, and a hose fitted to the lid, the other tip was immersed in a container with water 20 cm high, for gases to be released.

During the experiment, the removal of weeds was manually performed with hoes. During periods of absence of precipitation, drip tape irrigation was provided, with a two-day irrigation shift. No phytosanitary control was necessary due to the absence of pests and diseases that caused economic damage.

The determination of chlorophyll *a*, *b* and total was performed at the beginning of the flowering stage (45 DAS) by collecting the fourth fully expanded leaf from the apex. The leaves were detached from each plant, packed in aluminum envelopes and stored at low temperature in thermal containers with dry ice and sawdust, with refrigeration maintenance for subsequent extraction and destructive quantification of photosynthetic pigments. Leaf discs (Ø = 1.6 cm) were taken from each leaf, the fresh mass was determined in an analytical scale of 0.0001 g precision. After weighted, the material was macerated and placed in aluminum-lined containers, with 25 ml of 80% acetone, under an environment with a low-intensity green artificial light source. The containers were refrigerated (8ºC) for 24 hours and, subsequently, were filtered on paper for 5 minutes. The extracts absorbances were read on a GENESYS 10S-Thermo Scientific® UV-Vis spectrophotometer, at wavelengths (λ) of 647 and 663 nm (Arnon, 1949). The levels of chlorophylls *a* and *b* in the reading solutions were obtained using the equations described by Lichtenthaler (1987): chlorophyll *a* = 12.25 * A663 - 2.79 * A647; chlorophyll *b* = 21.50 * A647 - 5.10 * A663. The values obtained were transformed to the chlorophyll *a* and *b* content levels in the leaves, expressed in mg g^{-1} fresh matter.

The gas exchange evaluations were carried out during the flowering stage (45 DAS), between 9 am and 11 am, on the fourth fully expanded leaf from the apex, using the infrared gas analyzer (IRGA), model LCPro + Portable Photosynthesis System® (ADC BioScientificLCpro-SD System Serial No.33078), with photosynthetic photon density of 1200 µmol photons m^{-2} s^{-1} and airflow of 300 ml min $^{-1}$. The physiological variables analyzed were liquid photosynthesis (A - expressed in µmol m^{-2} s^{-1}), stomatal conductance (gs - mol m^{-2} s^{-1}), transpiration (T - mmol m^{-2} s^{-1}), internal CO_2 concentration (Ci - µmol mol^{-1}). Through the relationship between liquid photosynthesis (A) and transpiration (T), instantaneous water use efficiency was obtained (iWUE) [(µmol m^{-2} s^{-1}) (mmol m^{-2} s^{-1}) $^{-1}$] and the instantaneous carboxylation efficiency (iCE) [(µmol m^{-2} s^{-1}) (µmol mol^{-1}) $^{-1}$], was obtained by the ratio A/Ci.

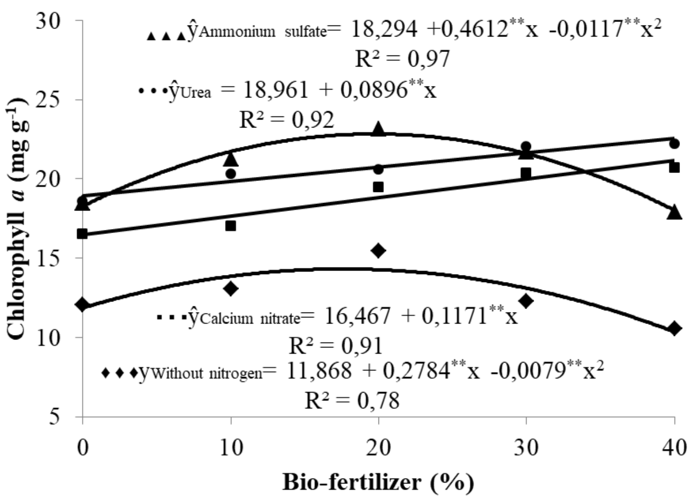
Data were submitted to analysis of variance by the F test, and the means compared by the Tukey ($p< 0.05$). The results related to the concentrations of cattle bio-fertilizer were submitted to regression analysis, the significant model with the determination coefficient (R^2) greater than 0.60 was selected. The software SAS (Statistical Analysis System, versão 9.2) was used.

3. Results and Discussion

The values of chlorophyll *a*, total chlorophyll, liquid photosynthesis, stomatal conductance, transpiration, internal carbon concentration, and the instantaneous carboxylation efficiency were influenced by the interaction between cattle bio-fertilizer concentrations and nitrogen sources (P <0.01), however, the chlorophyll content and the instantaneous water use efficiency responded in isolation to the effects of nitrogen sources.

The chlorophyll *a* levels under the bio-fertilizer concentrations of 20 and 18% combined with ammonium sulfate and without the use of nitrogen responded in a quadratic manner with maximum values of 23 and 14 mg g^{-1} MF, respectively. For calcium nitrate and urea, a linear increased, with values of 17 and 23 mg g^{-1} MF, respectively, provided by the highest concentration of 40% of the bio-fertilizer was observed (Figure 1).

Figure 1. Chlorophyll *a* content in zucchini plants fertilized with different concentrations of bio-fertilizer and nitrogen sources.



Source: Authors.

Considering the cultivation conditions and the chlorophyll *a* level, with the increase of the bio-fertilizer concentration, an increasing tendency of this photosynthetic pigment was observed, regardless of the nitrogen source used. The lowest levels of chlorophyll *a* were observed in plants grown without nitrogen (14 mg g^{-1} MF) and with calcium nitrate (17 mg g^{-1} MF), yet, the levels were always above 10 mg g^{-1} MF, which was expected considering the need for nitrogen and magnesium for pigment synthesis, and the fact that they are also present

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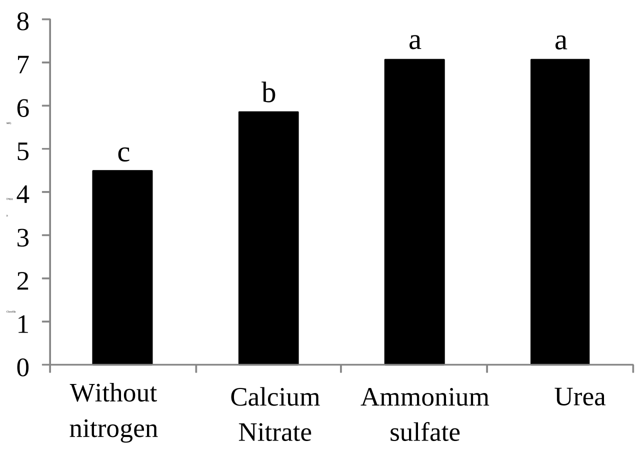
in the bio-fertilizer composition (N = 0.45 g L^{-1} and Mg $^{+2}$ = 0.13 g L^{-1}). In cucurbits, the incorporation of N, up to a certain limit, causes an increase in leaf area, consequently, it has an effect on the production of photoassimilates (Pôrto et al., 2014; Purquerio et al., 2019).

The highest levels were observed with ammonium sulfate when the concentration of the bio-fertilizer was 20% and showed a decrease with higher concentrations of the bio-fertilizer, followed by the treatment with urea, that showed a non-linear response tendency. Urea may be less effective than ammonium sulfate, due to the change in soil pH by ammonium sulfate increasing the availability of other nutrients (Silva et al., 2017).

Chlorophyll is the main responsible for the photochemical reaction of photosynthesis in plants, this organic molecule also retains most of the leaf nitrogen. Therefore, an increase in the concentration of this molecule in response to increasing nitrogen supply has been reported in several studies on other crops (Pôrto et al., 2014). For the chlorophyll *b* content, no interaction between the bio-fertilizer and the different sources of nitrogen was observed.

However, when the chlorophyll *b* levels were compared, it was found that the best sources used were ammonium sulfate (7.07 mg g^{-1} MF) and urea (7.06 mg g^{-1} MF), which did not differ from each other, but were statistically different and higher than the values provided by calcium nitrate (5.84 mg g^{-1} MF) and without the use of nitrogen (4.48 mg g^{-1} MF) (Figure 2).

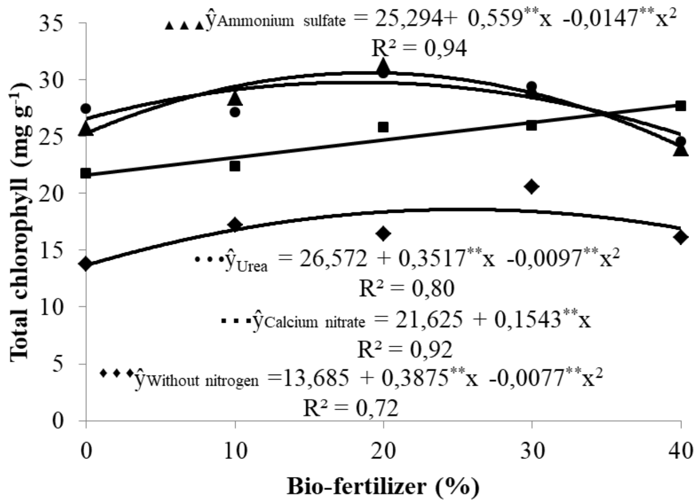
Figure 2. Means of chlorophyll *b* content in zucchini plants fertilized with nitrogen sources. DMS = 1.04.



Source: Authors.

The highest mean value of 30 mg g^{-1} MF of total chlorophyll was provided by the ammonium sulfate source at 20% of the bio-fertilizer and with urea at a maximum concentration of 40% of the bio-fertilizer, with calcium nitrate and without nitrogen, the values of 28 mg g^{-1} MF and 19 mg g^{-1} MF were observed, respectively (Figure 3). The results obtained were superior to those observed by Pôrto et al. (2014), in which fertilization of zucchini and cucumber with 331 and 417 kg ha^{-1} of N, provided a total chlorophyll content of 5.12 and 6.02 mg g^{-1}.

Figure 3: Total chlorophyll content in zucchini plants fertilized with different concentrations of bio-fertilizer and nitrogen sources.



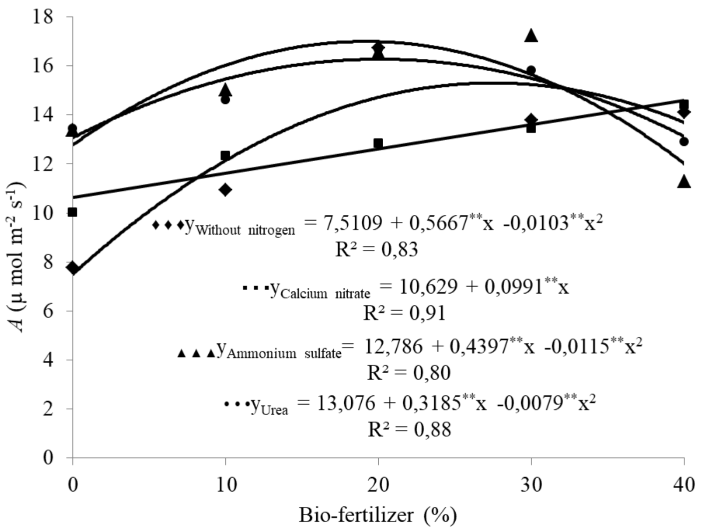
Source: Authors.

Plants generally respond well to nitrogen fertilization, with the most visible external effect being the green color of leaves. Nitrogen is considered an essential macronutrient, and plays an important role in several metabolic functions in plant cells, including constituents of the chlorophyll molecule (Silva et al., 2017). Its low availability in the soil, and consequently in the leaf tissue, induce yellowing of the leaves, a symptom of pigment absence.

Therefore, adequate nitrogen nutrition leads to the synthesis of chlorophyll, considering that nitrogen fertilization is the main responsible for the availability of this nutrient in the soil. The comparative analysis of the chemical properties of the different nitrogen sources suggests that the greater solubility of calcium nitrate (121 g.100 mL^{-1}) compared to the other sources used (100 g.100 mL^{-1} and 73 g.100 mL^{-1}, for urea and ammonium sulfate, respectively) may have favored their leaching and consequently induced a lower nitrogen availability for zucchini plants over time. Also, the presence of sulfur plays an important role in protein and enzyme metabolism, which constitutes acetyl-CoA and ferredoxin (molecule involved in the process of N_2 fixation, nitrate reduction and electron transport in photosynthesis) (Taiz et al., 2015).

The liquid photosynthesis (A) content estimated by derivative were 17, 16 and 15 µ mol m^2 s^{-1} at concentrations of 19, 20 and 28% of bio-fertilizer combined with ammonium sulfate, urea and without nitrogen, and through a linear model adjustment of the bio-fertilizer concentrations and the use of calcium nitrate, it was found that the maximum value of A was 15 µ mol m^2 s^{-1} combined with the maximum concentration of 40% of bio-fertilizer (Figure 4). The results obtained with the use of bio-fertilizer and nitrogen sources were higher than the value of 10.12 µ mol m^2 s^{-1} found by Freire et al. (2014), in passion fruit plants irrigated with high salinity water, with bio-fertilizer, and without mulch.

Figure 4. Liquid photosynthesis (A) in zucchini fertilized with concentrations of cattle bio-fertilizer and nitrogen sources.

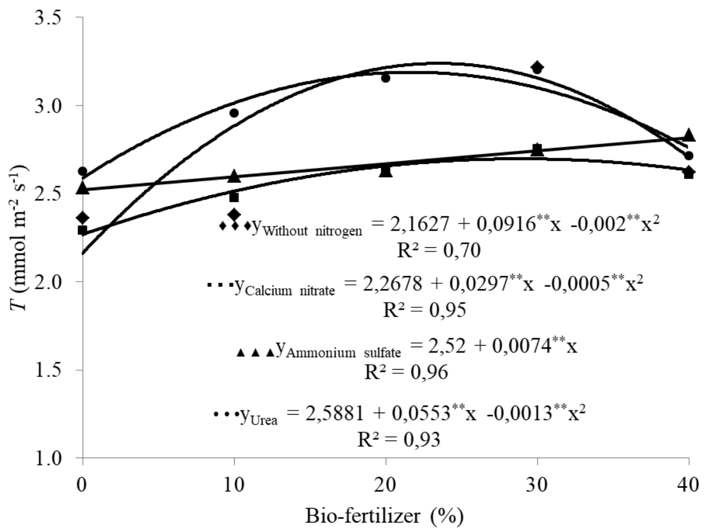


Source: Authors.

The results obtained show an increased response as the concentration of the bio-fertilizer increased regardless of the nitrogen source used, up to 28% of bio-fertilizer, from which it decreased, except for the calcium nitrate source, which showed a linear response. The concentration that best stimulated photosynthesis (17 µ mol m^2 s^{-1}) was 19% of bio-fertilizer with ammonium sulfate followed by urea (16 µ mol m^2 s^{-1}) at a concentration of 20%, similar results were also obtained for the levels of chlorophyll *a*, *b* and total, which provided higher photosynthetic levels with the association of bio-fertilizer with nitrogen sources. Cattle bio-fertilizer, is a source of bioactive compounds, that exerts a positive action on plant nutrition and stimulates the release of humic substances in the soil, favoring greater water absorption and accumulation of nutrients by plants, it also stimulates cell division and development, and provides greater nitrogen accumulation in plants (Silva et al., 2019).

Transpiration of 2.8; 3.2; 2.7 and 3.2 mmol m^2 s^{-1} were obtained using ammonium sulfate, urea, calcium nitrate and no nitrogen associated with 40, 21, 30 and 23% bio-fertilizer, respectively (Figure 5). The highest mean values of transpiration (3.2 mmol m^2 s^{-1}) were found with zucchini plants fertilized with urea and only the use of bio-fertilizer. Shimazaki et al. (2007) emphasized that water loss by plants is regulated by the activity of guard cells and the increase in plant transpiration, mainly due to the inability of some plants to absorb enough water to replace that consumed in the transpiratory process. Some studies have suggested that an increased application of N can improve the water use efficiency and avoid membrane damage in the cell by improving osmoregulation (Cossani et al., 2012; Carvalho et al., 2016). Nevertheless, in this study, calcium nitrate and the use of bio-fertilizer alone did not efficiently reduce the transpiration of zucchini plants.

Figure 5. Transpiration (T) in zucchini plants fertilized with concentrations of bio-fertilizer and nitrogen sources.



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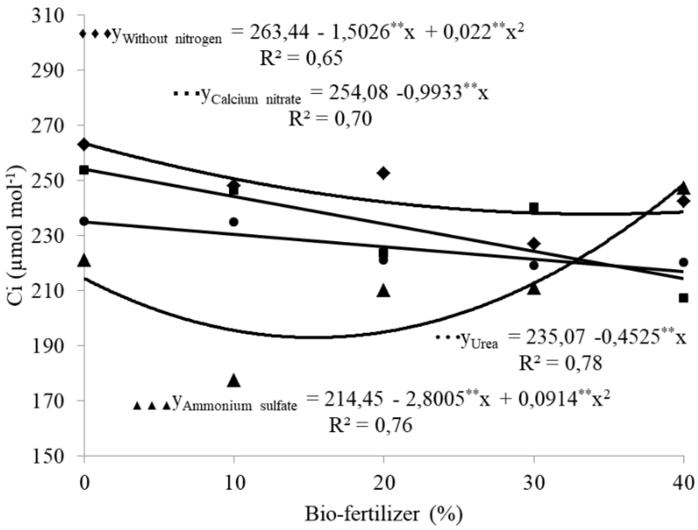
The treatments with ammonium sulfate and without nitrogen associated with bio-fertilizer concentrations of 15 and 34% provided internal carbon concentrations (C_i) of 193 and 238 μmol mol^{-1}, respectively. Under the maximum concentration of 40% of bio-fertilizer with urea and calcium nitrate, the values of 217 and 214 μmol mol^{-1} were obtained, respectively (Figure 6). Freire et al. (2014), observed higher C_i in passion fruit plants fertilized with bio-fertilizer than those that did not receive the organic input, and an increase from 206.2 to 229.3 μmol mol^{-1} of C_i was observed, similar to the result obtained with the use of bio-fertilizer alone (238 μmol mol^{-1}), the highest value observed for C_i.

Figure 6. Internal CO_2 (C_i) concentration in zucchini plants fertilized with concentrations of bio-fertilizer and nitrogen sources.



Source: Authors.

The values of C_i considered high suggests that CO_2 is not being used for the synthesis of carbohydrate in the photosynthetic process (Freire et al., 2014), which was confirmed with the results obtained without the use of nitrogen, since the higher the value for Ci lower was the net photosynthesis recorded (Figure 4). The C_i in the leaf mesophyll is reduced by stomatal closure, thus, there is a restriction on the entry of CO_2 into the cells, which could increase the susceptibility to photochemical damage, because low rates of CO_2 assimilation cause excessive light energy in the FSII (Freire et al., 2014). According to Ferraz et al. (2015) the increase in C_i may be directly related to transpiration, as demonstrated by the highest C_i values (238 and 217 μmol mol^{-1}) provided by urea and the use of bio-fertilizer alone.

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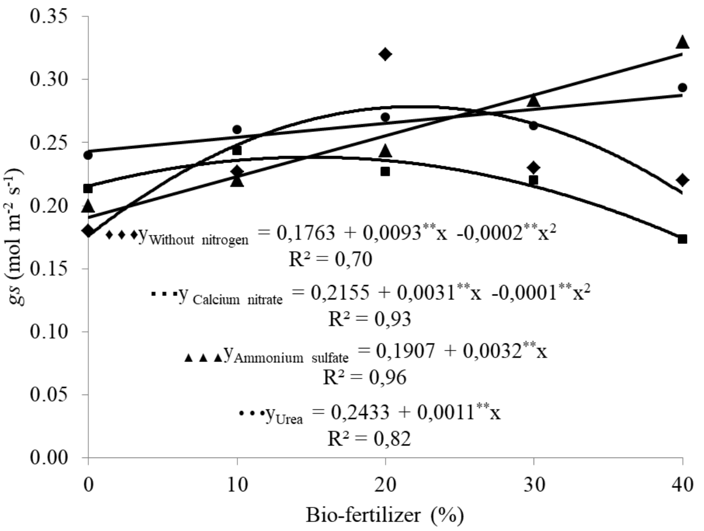
It was observed that the concentration of 40% provided stomatal conductance (*gs*) of 0.32 and 0.2 mol m^2 s^{-1} associated with ammonium sulfate and urea, respectively. For the treatments with calcium nitrate and without nitrogen, 0.24 and 0.28 mol m^2 s for *gs* were recorded, respectively, under the concentrations of 16 and 23% of bio-fertilizer (Figure 7). Even in the absence of nitrogen sources, there was an increase in *gs* when the zucchini plants were fertilized only with bio-fertilizer. Freire et al. (2014), highlights the importance of organic input in the soil to improve the water content in the soil and, consequently, to the plants, enabling gas exchange between the plants and the environment.

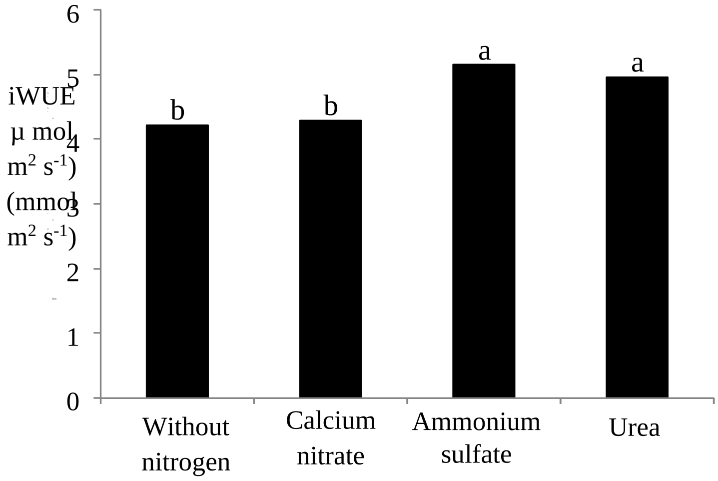
Figure 7. Stomatal conductance (*gs*) in zucchini plants fertilized with concentrations of bio-fertilizer and nitrogen sources.



Source: Authors.

Among the nitrogen sources, ammonium sulfate provided the highest instantaneous water use efficiency iWUE with the value of 5.14 (µ mol m^2 s^{-1}) (mmol m^2 s^{-1}) $^{-1}$, however it did not differ statistically from the urea treatment [4.95 (µ mol m^2 s^{-1}) (mmol m^2 s^{-1}) $^{-1}$], this was higher than that obtained for calcium nitrate and without nitrogen with the values of 4.28 and 4.21 (µ mol m^2 s^{-1}) (mmol m^2 s^{-1}) $^{-1}$, which were similar (Figure 8). When the water available in the soil decreases, through the control of stomata opening plants increase the iWUE to minimize water losses as a strategy. When the different sources of nitrogen were compared, it was found that the most efficient reduction in water loss was observed in zucchini under the treatments with ammonium sulfate and urea.

Figure 8. Instantaneous water use efficiency (iWUE) in zucchini plants fertilized with nitrogen sources. DMS = 0.56.

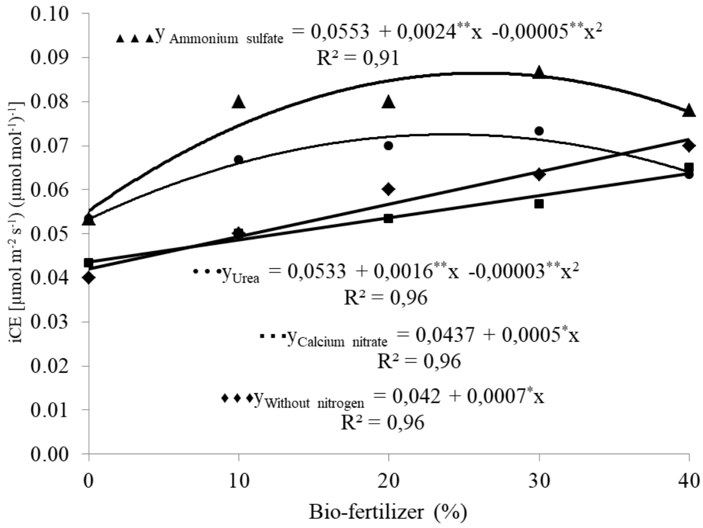


Source: Authors.

The reduction in stomatal conductance is a response of plants towards stress conditions to reduce water loss (Melo et al., 2017). As a consequence, the water use efficiency increases with the reduction of stomatal conductance, since the use of water molecules becomes more efficient (Campos et al., 2014). The efficient use of water involves maximum moisture retention of the soil for transpiration, also involving reduced non-stomatic transpiration and minimum water loss by evaporation of the soil, resulting in improvements in water condition and yield success. As water availability increases, the crop can better express its productive potential by increasing the photosynthetic capacity (Melo et al., 2017). Therefore, the lowest value of iWUE observed in zucchini plants with the use of bio-fertilizer alone and calcium nitrate is probably due to the increases in evapotranspiration and Ci (Figures 5 and 6).

The concentrations of 18 and 27% of bio-fertilizer provided the values of instantaneous carboxylation efficiency (iCE) of 0.09 and 0.07 (μmol m^{-2} s^{-1}) (μmol mol^{-1}) $^{-1}$, respectively, under the treatments with ammonium sulfate and urea. With the increase in the concentrations of bio-fertilizer and calcium nitrate and without the use of nitrogen, the values of 0.06 and 0.07 (μmol m^{-2} s^{-1}) (μmol mol^{-1}) $^{-1}$ (Figure 9) were obtained respectively, at the concentration of 40%. Considering the cultivation conditions and the results obtained, with the increase in the bio-fertilizer concentrations, an increasing tendency of iCE was observed, independently of the nitrogen source used. However, the highest value was observed when the zucchini plants were fertilized with ammonium sulfate and the concentration of 18% of the bio-fertilizer, as observed for A and Ci (Figures 4 and 6), where the best results were obtained with the ammonium sulfate.

Figure 9. Instantaneous carboxylation efficiency (iCE) in zucchini plants fertilized with nitrogen sources.



Source: Authors.

The iCE is used to study the non-stomatal factors that interfere with the photosynthetic rate and is related to the A and Ci rates inside the sub-stomatal chamber (Ferraz et al. 2015). If the Ci values are too low, the entry of CO_2 into the mesophyll cells becomes limited. Thus, the plant uses CO_2 from respiration to maintain a minimum level of photosynthetic rate (Melo et al. 2017). Based on the results, a decrease was observed in the iCE of the zucchini plants fertilized with urea [(0.07 µmol m^{-2} s^{-1}) (µmol mol^{-1}) $^{-1}$], without nitrogen [(0.07 µmol m^{-2} s^{-1}) (µmol mol^{-1}) $^{-1}$] and calcium nitrate [(0.06 µmol m^{-2} s^{-1}) (µmol mol^{-1}) $^{-1}$].

Nitrogen is a essential nutrient in several stages of the photosynthetic process and gas exchange in zucchini plants. Based on the results obtained, the best sources of nitrogen were ammonium sulfate and urea associated with the bio-fertilizer up to the concentration of 20%.

4. Final Considerations

Ammonium sulfate and urea were the sources of nitrogen that favored the greatest accumulation of chlorophyll *b* and the greatest instantaneous water use efficiency. The combination of ammonium sulfate and bio-fertilizer provided the best performance for the photosynthetic process and the hydric process of the plants. Application of the bio-fertilizer alone does not replace any nitrogen source.

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