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GAS EXCHANGES AND CHLOROPHYLL CONTENT IN GREEN PEPPER PLANTS UNDER BIO-FERTILIZATION AND TIMES OF APPLICATION

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Abstract
This study aimed to determine the gas exchange and the chlorophyll content of green pepper plants under doses and times of application of bio-fertilizers based on manure and enriched organic compost. Two experiments were carried out simultaneously with applications of bio-fertilizers prepared from manure and enriched organic compost, one using cattle manure (CBF) and the other sheep manure (SBF). For these, four doses of biological fertilizers (100, 200, 300 and 400 dm³ ha-1), three application times (0, 30 and 60 days after transplantation - DAT) and absolute control, referring to the absence of fertilization, were used. treatments. were arranged in a randomized block design, totaling 13 treatments. The variables evaluated were: the relative chlorophyll *a*, *b* and total content; liquid photosynthesis (A); stomatal conductance (gs); internal CO_2 concentration (Ci); instant carboxylation efficiency (iCE - A/Ci); transpiration rate (T); intrinsic water use efficiency (iWUE - A/gs); and water use efficiency (WUE - A/E). Gs, A and T, showed significant effect at 60 DAT with the application of SBF and Ci at 30 DAT with CBF. The dose of 400 dm³ ha^{-1} of SBF provided greater gas results, and the doses of 200 and 300 dm³ ha^{-1} of CBF promoted a greater Ci, greater stomatal conductance, greater liquid photosynthesis and better water use efficiency, which results in a greater plant fresh weight at the time of flowering induction.

Keywords: *Capsicum annuum*. Organic Input. Photosynthesis. Stomatal Conductance.

1. Introduction

Gas exchanges are very important to maintain plant vital processes, such as nutrients transport, the production of photoassimilates, cell expansion, cooling and plant cell water regulation. For these events to happen, the characteristics of the soil must provide good conditions for the plants (Cavalcante et al. 2019).

Bio-fertilizers that have animal residues in their composition, they promote benefits to of the soil. They act as soil conditioners, fertilizer, pH corrector, and microbiological inoculant, in addition to its low cost they can be easily found (Celedonio et al. 2016; Matos et al. 2018).

Bio-fertilizers are rich in nutrients, organic matter, and microorganisms. These enable greater availability of nutrients and plant yield performance, through the decomposition of organic matter. It also provides benefits to the soil, promoting higher fertility, greater water retention by less evaporation loss, due to the formation of a layer of organic matter on its surface (Guimarães et al. 2017).

Thus, the application of biological fertilizers or bio-fertilizers can influence their gas exchange and the production of chlorophylls, this occurs, due to the nutritional composition of bio-fertilizers, which is rich in potassium (Khafagy et al. 2019). Potassium is an activator of enzymes involved in plant photorespiration, in addition, it is the main cation that establishes cell turgor and the maintenance of electroneutrality, promoting the regulation of the osmotic potential of plant cells (Taiz et al. 2017).

This study aimed to determine the gas exchange and chlorophyll *a*, *b* and total content in green pepper leaves under doses and application times of manure-based bio-fertilizers and enriched organic compost.

2. Material and Methods

The experiment was carried out between February and May of 2018, in a commercial area at the Irrigated perimeter of Apolônio Sales, Pernambuco. Located between the 380 13' and 380 18' W meridians and the 80 53' and 90 00' S parallels, near the São Francisco River, occupying an area of 3,845 ha, in which 808 ha is exclusive for irrigated agriculture. The local climate is of the BSh type, based on the Köppen and Geiger classification, with a thermal average of 26 °C, an annual rainfall average of 425 mm and 2.385.6 mm of annual evaporation (Embrapa 2004; Alvares et al. 2014).

The average daily temperature and relative humidity of the experimental area were recorded with a TH-500 digital thermohygrometer device, and the rainfall with the TFA 4760 model (Figure 1).

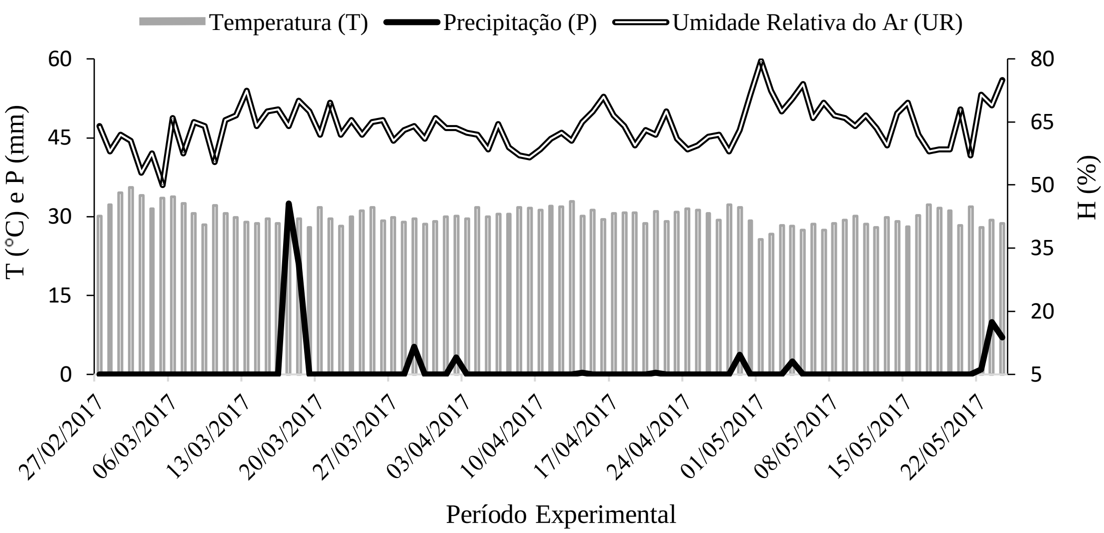

Figure 1. Meteorological conditions during the experimental period.

Seeds of hybrid cv Solário green pepper from Clause Vegetable Seeds were used. The seeds were sown in 128-cell trays, containing commercial substrate Bioplant®, and placed in a greenhouse for germination and seedling emergence where a micro-sprinkling system was used for irrigation. The transplant was carried out 32 days after sowing (DAS) in a bed with dimensions of 1.5 x 0.5. At 37 DAT, 80 kg ha^{-1} of urea was applied, at five DAT, 130 kg ha^{-1} of the formulation 06-24-12 and 260 kg ha^{-1} of the formulation 20-10-20 at seven DAT based on soil fertilization recommendations by Cavalcante (2008). The experimental plot consisted of four-meter rows, with 32 plants. The useful area consisted of two central rows, corresponding to six m^2, totaling eight plants.

The soil used was an Oxisolic Quartzenic Neossol (Embrapa 2006) and presented low fertility and low moisture retention capacity (Table 1). The total organic carbon of cattle manure (CBF) and sheep manure (SBF) had values of 8.60 and 6.23 g kg-, respectively.

Table 1. Chemical attributes of the soil.

Soil sample	pH	P	K^+	Na^+	H^++Al^{+3}	Al^{+3}	Ca^{+2}	Mg^{+2}	SB	CTC	V	m	O.M
	H_2O (1:2.5)	mg dm^{-3}		cmol$_c$ dm^{-3}							%		g kg^{-1}
CBF	6.95	388.01	152.88	0.12	3.47	-	3.50	1.50	5.51	8.98	61.40	-	10.73
SBF	6.81	497.50	155.22	0.10	3.14	-	3.50	1.10	5.10	8.23	61.92	-	14.83

$H^{++}Al^{+3}$: Potential acidity; SB: Sum of bases; CTC: Cation exchange capacity; V: Base saturation; m: Aluminum saturation; O.M.: Organic matter; CBF: Biological fertilizer prepared with cattle manure; SBF: Biological fertilizer prepared with sheep manure.

Two types of biological fertilizers were prepared for use in the experiments, one based on cattle manure (CBF) and the other based on ear manure, both with enriched organic compost. Four doses of biological fertilizers (100, 200, 300 and 400 dm^3 ha-1), three times of application (0, 30 and 60 days after transplantation - DAT) and absolute control, referring to the absence of fertilization, were used for these treatments. were arranged in a design using randomized blocks, totaling 13 treatments.

The application of biological fertilizers was structured as follows: integral application in the transplant (0 DAT), treatments from 1 to 4; divided into two applications (0 and 30 DAT), treatments from 5 to 8; and divided into three applications (0, 30 and 60 DAT), treatments from 9 to 12 (Table 2).

Table 2. Treatments used in the experiment.

Treatments	Times of application (DAT)	0	30	60
		Doses dm^3 ha^{-1}		
1	0	100.0		
2	0	200.0		
3	0	300.0		
4	0	400.0		
5	30	50.0	50.0	
6	30	100.0	100.0	
7	30	150.0	150.0	
8	30	200.0	200.0	
9	60	33.33	33.33	33.33
10	60	66.66	66.66	66.66
11	60	100.0	100.0	100.0
12	60	133.33	133.33	133.33
13	Absolute control	0.0	0.0	0.0

Biofertilizers were prepared in two biofactories with a capacity of 100 dm3. Each biofactory was filled with 5 kg of enriched organic compost, 15 dm3 of manure (cattle or sheep) and the remaining volume supplemented with untreated water, corresponding to the proportions of 5 and 15% of enriched organic compost and manure, respectively. Biological fertilizers were stirred every three days and were ready for use 15 days after preparation (Microgeo 2019) (Table 3).

Using a 5 dm3 manual sprayer, the application of biological fertilizers was carried out, previously filtered in screen of mesh of 2 mm.

Table 3. Chemical analysis of the bio-fertilizers.

Bio-fertilizer	H	CO	N	P	K	Ca	Mg	S	Na	Cu	Zn	Fe	Mn	B
	%		g L^{-1}							mg L^{-1}				
CBF	98.7	10.4	0.1	0.08	30.9	0.22	0.58	0.05	-	0.43	0.61	5.12	0.62	2.22
SBF	98.8	11.1	0.11	0.07	24.3	0.15	0.27	0.07	-	0.46	0.54	10.6	0.51	2.13

CBF: Biological fertilizer prepared with cattle manure; SBF: Biological fertilizer prepared with sheep manure; H: humidity; CO: Dichromate oxidation method; N, P, K, Ca and Mg: Digestion with H_2O_2 and H_2SO_4; S, Fe, Cu, Mn, Zn, and Na: Digestion with HNO_3 $HClO_4$; B: Dry combustion extraction.

Irrigation was performed by dripping with the aid of an electronic soil moisture meter (Model HidroFarm HFM2030). Irrigation was divided into two applications 4.56 mm in the morning and 4.56 mm in the late afternoon (Table 4). On days when there was enough rain, irrigation was suspended and supplemented when necessary.

Table 4. Chemical attributes of water used in irrigation.

pH	C.E.	SO_4^{-2}	Mg^{2+}	Na^+	K^+	Ca^{2+}	CO_3^{-2}	HCO_3^{-2}	Cl	SAR	PES	Classification
	$dS\ m^{-1}$	$mg\ L^{-1}$			$mmol_c\ L^{-1}$							
6.7	0.09	0	0.18	0.2	0.1	0.1	0	1.2	0.3	0.6	0	C_1S_1

E.C.: Electrical conductivity at 25 °C; SAR: Sodium adsorption ratio; PES: Percentage of exchangeable sodium. C_1S_1: Richards (1954).

The physiological evaluations were carried out at the beginning of the flowering stage (31 DAT) and were determined: the relative content of chlorophyll *a*, *b* and total, using the portable chlorophyll meter, model ClorofiLOG CFL1030; The gas exchange variables: liquid photosynthesis (A); stomatal conductance (gs); internal CO_2 concentration (Ci); instant carboxylation efficiency (iCE - A/Ci); transpiration rate (T); intrinsic water use efficiency (EiUA - A/gs); and water use efficiency (WUE - A/T), using the infrared gas analyzer (IRGA, LI-COR - model LI-6400XT Portable Photosynthesis System). The readings were made on the young leaf of the fully expanded leaf, between 7 am and 11 am.

Data were submitted to the test of normality (Shapiro-Wilk) and homogeneity (Bartlett), then subjected to analysis of variance, with no significant differences by the F test. However, statistical analyzes were performed with their interval of confidence to compare the two types of biological fertilizers, in this way 100 confidence intervals were generated between the minimum and maximum values using the statistical program R (R Core Team 2018).

3. Results and Discussion

The application times showed an effect for stomatal conductance (gs), liquid photosynthesis (A), transpiration rate (T) and internal carbon concentration (Ci). For gs, A and T, SBF showed superior results in relation to ABB in the application at 60 days after transplantation (DAT), different from the Ci in which CBF was superior in all application periods (Figure 2A-D).

In general, the results obtained for the physiological characteristics, stomatal conductance (gs), transpiration (T) and internal carbon concentration (Ci) in the conditions of this experiment, suggest that the use CBF between 45 to 57 DAT (Figure 2 A, C, and D), allowed an adequate supply of nutrients in the photosynthetic processes, enhancing the photosynthetic apparatus and thereby ensuring efficiency in the gas exchange characteristics of the plants.

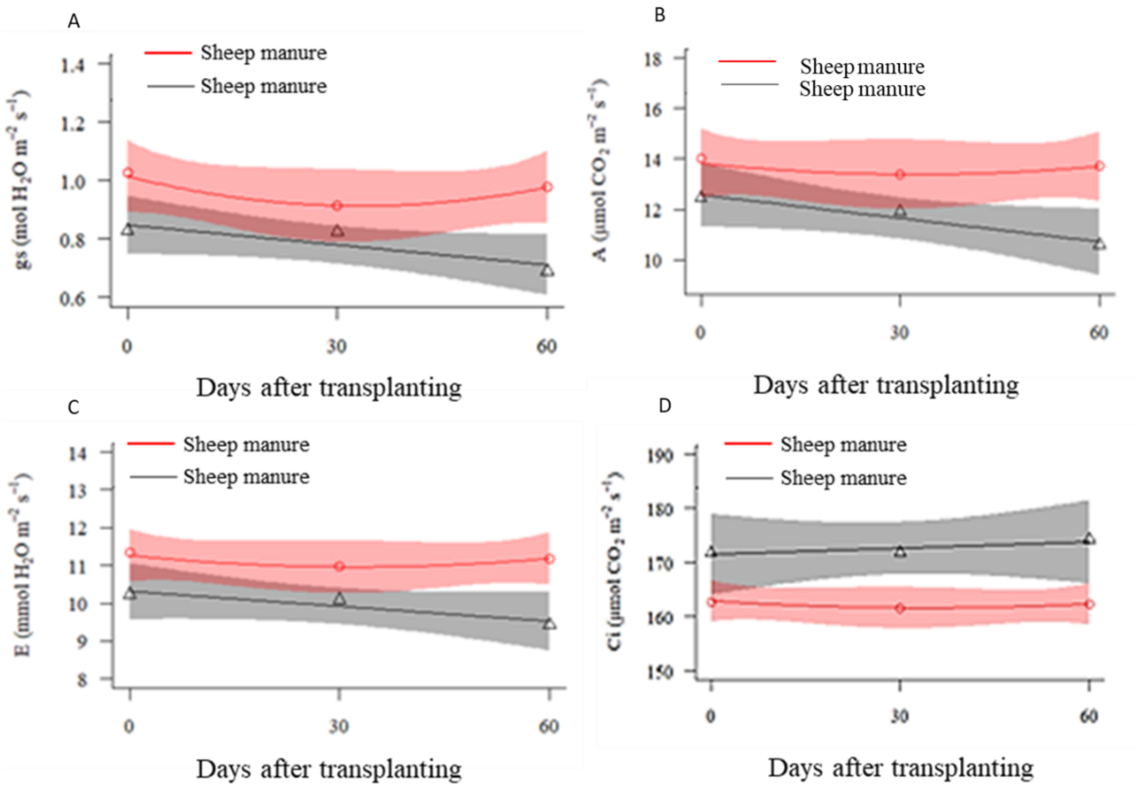

Figure 2. A – stomatal conductance (gs) in green pepper plants under bio-fertilization and application times (Sheep manure and Cattle manure); B – liquid photosynthesis (A) in green pepper plants under bio-fertilization and application times (Sheep manure and Cattle manure); C – transpiration (E) in green pepper plants under bio-fertilization and application times (Sheep manure and Cattle manure); D – internal carbon concentration (Ci) in green pepper plants under bio-fertilization and application times (Sheep manure and Cattle manure).

Several environmental factors influence the speed of the photosynthetic process, such as the best use of solar radiation, CO_2, temperature, oxygen, water, fertilizers, and others. Such abiotic factors can better explain the physiological properties in C_3 plants. According to Taiz et al. (2017) C_3 plants should exhibit a net photosynthetic rate between 10 and 25 µmol m^{-2} s^{-1}. The value obtained for the net photosynthetic rate in the present study with the addition of SBF biofertilizers for all application times was approximately 14 µmol m^{-2} s^{-1} (Figure 2B), this value is considered satisfactory since the green pepper cv Solário is a C_3 plant. Similar responses were detected at 0 and 30 DAT with the addition of CBF when at 60 DAT CBF showed net photosynthesis of approximately 11 µmol m^{-2} s^{-1} (Figure 2B) which is also considered satisfactory for C_3 plants.

Sousa et al. (2014) evaluated the effect of biofertilizers on gas exchange of cowpea beans under saline stress and observed that the enriched crab biofertilizer attenuated the effects of salt stress on A, T and gs when compared to plants that did not receive biofertilizer and plants fertilized with common cattle biofertilizers, probably due to the greater amount of essential nutrients and the better osmotic adjustment

Stomatal conductance, liquid photosynthesis and transpiration were higher in the SBF, with emphasis on the dose of 400 Biofertilizer dose dm^3 ha^{-1}, while for the internal carbon concentration the highest averages were observed with application of CBF (Figure 3A-D).

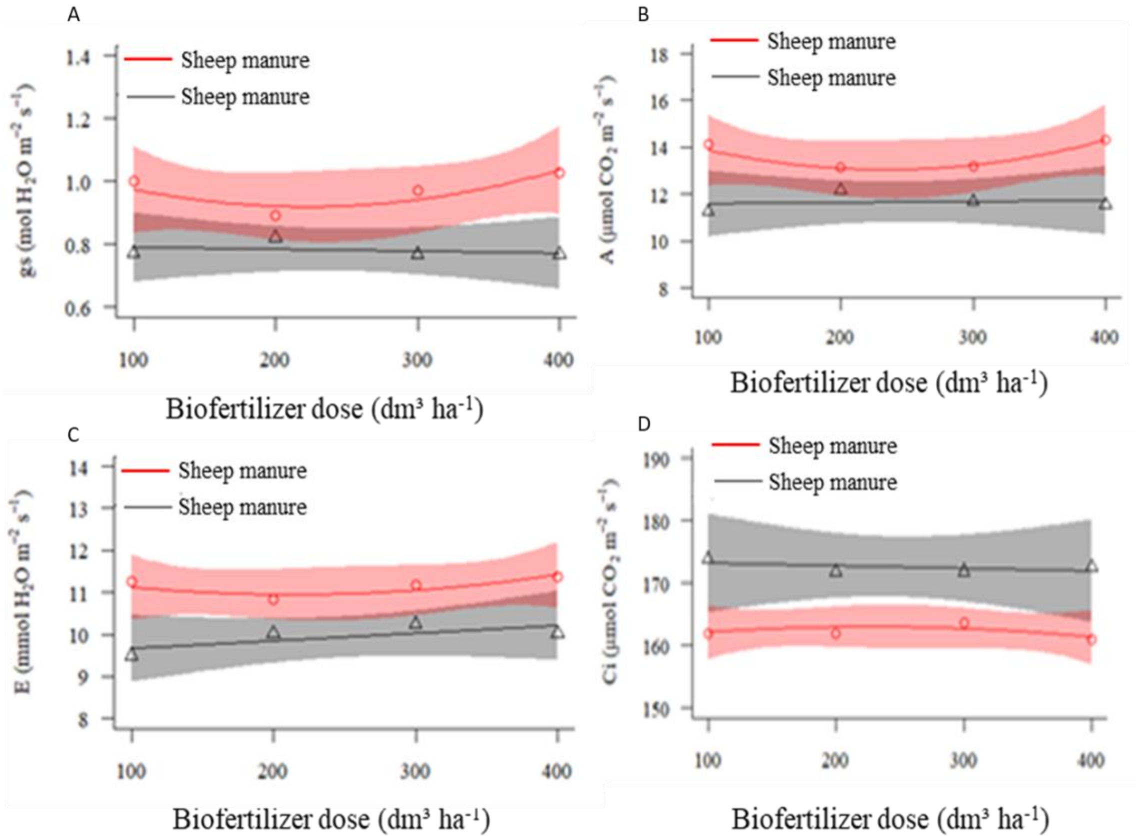

Figure 3. A – stomatal conductance (gs) in green pepper plants under bio-fertilizers in different doses (Sheep manure and Cattle manure); B – liquid photosynthesis (A) in green pepper plants under bio-fertilizers in different doses (Sheep manure and Cattle manure); C – transpiration (T) in green pepper plants under bio-fertilizers in different doses (Sheep manure and Cattle manure); D – internal carbon concentration (Ci) in green pepper plants under bio-fertilizers in different doses (Sheep manure and Cattle manure).

This occurs possibly due to the adequate doses of nutrients (Table 3) provided, such as N, phosphorus (P) which allows the efficient functioning of the electron transport chain, and being a component of adenosine triphosphate (ATP) supports the adequate production of this molecule for optimal levels of CO_2 fixation (Carstensen et al. 2018) thus promoting high iEC, as observed for SBF (Figure 4A).

After 45 DAT, the instant efficiency of carboxylation (iEC) showed a difference between the biological fertilizers (Figure 4A). iEC can be understood as the level of carbon use available in the sub-stomatal chamber for fixing CO_2. Thus, the higher values of iEC, in plants fertilized with (SBF), may be the result of both the optimal levels of Ci (a factor considered of stomatic nature), as well as the adequate supply of nutrients (Table 3) which participate in the formation, maintenance and activation of compounds related to photosynthesis (non-stomatal factors).

As mentioned before, optimal levels of Ci and IEC are also related to stomatic nature, among which we can mention stomatal conductance (gs). Significant increases in gs as observed in figure 3 A, provide greater gas exchange, which means greater transpiration (E) and CO_2 influx in the leaf mesophile (Ci), thus enabling greater liquid photosynthesis (A) (Figure 3B) and consequently higher iEC (Figure 4A).

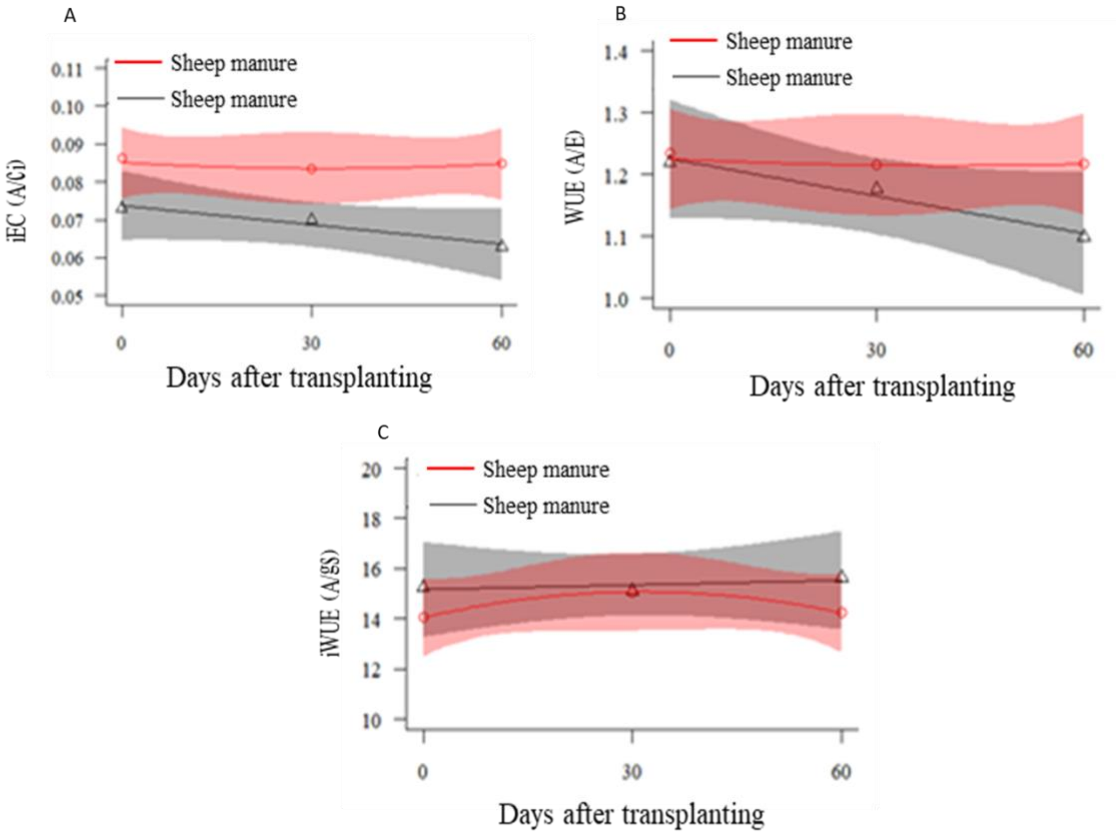

Figure 4. A – intrinsic carboxylation efficiency (iEC) of green pepper plants under biofertilization and application times (Sheep manure and Cattle manure); B – water use efficiency (WUE) of green pepper plants under biofertilization and application times (Sheep manure and Cattle manure); C – instant water use efficiency (iWUE) of green pepper plants under biofertilization and application times (Sheep manure and Cattle manure).

The water use efficiency (WUE) and the intrinsic efficiency of water use (iWUE) did not differ. It was observed that as the CBF application period increased, there was a decrease in iEC, WUE and an increase in iWUE (Figure 4A-C). This indicates that the hybrid pepper cv Solário managed to fix, more carbon per unit of water lost at both 30 DAT and 60 DAT. Similarly, there was an increase as the CBF doses increased (Figure 5 A-C). It is assumed that the concentration of K in the CBF bio-fertilizer (Table 3) was inadequate for the green pepper cultivation, thus causing the extraction of the malate from the guard cells, causing an efflux of K. Therefore, the stomata became slow and water vapor was lost, subsequently there was a decrease in iEC.

Adequate levels of K are extremely important for the crop because it regulates the activity of guard cells, decreasing water loss through transpiration, and consequently, increases the instant efficiency of carboxylation and the water use efficiency (Hasanuzzaman et al. 2018), a fact that occurred for SBF (Figure 5 A-C).

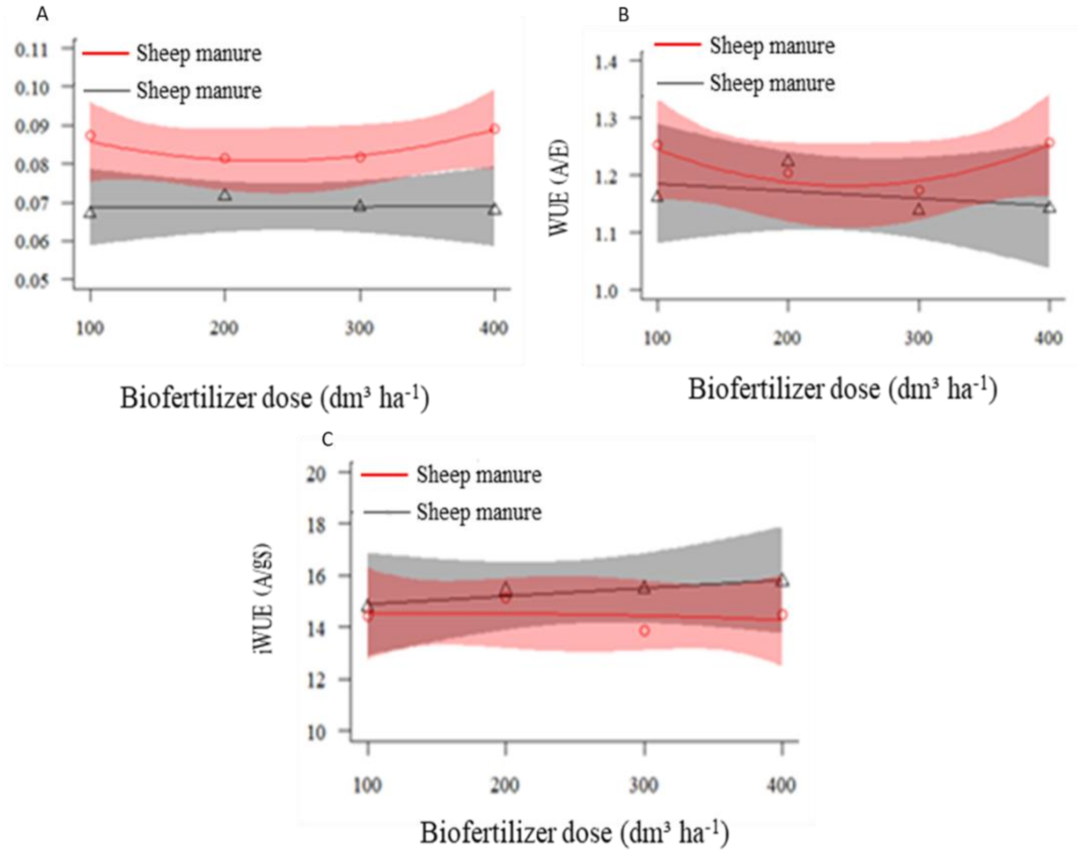

Figure 5. A – intrinsic carboxylation efficiency (iCE) obtained from green pepper plants under biofertilization in different doses (Sheep manure and Cattle manure); B – water use efficiency (WUE) obtained from green pepper plants under biofertilization in different doses (Sheep manure and Cattle manure); C – instant water use efficiency (iWUE) obtained from green pepper plants under biofertilization in different doses (Sheep manure and Cattle manure).

The chlorophyll *a* and *b* content and the chlorophyll *a/b* ratio did not differ between the biofertilizers at the time of application (Figure 6A, B, and D), while the total chlorophyll content (Figure 6 C) showed a difference only in the application performed at 0 DAT. However, it was noted for the application of SBF that the greater the number of days after transplantation, the chlorophyll *a, b* and *total* content increased, and the opposite occurred for the chlorophyll *a/b* ratio (Figure 6A, B, and C).

The results obtained for chlorophylls with the application of SBF agree with the higher values found for the physiological characteristic IEC, since the supply of the ideal amount of nutrients for the green pepper plants cv Solário favors the biosynthesis of chlorophyll molecules, thus, ideal concentrations of nutrients in the plant provides greater availability of substrate for chlorophyll synthesis.

On the other hand, CBF showed the opposite of what was observed for SBF (Figure 6 A, B and C), possibly due to the different composition between them. Similarly, Machuca (2018) in a study with green peppers under different irrigation depths did not find differences between chlorophylls *a* and *b*.

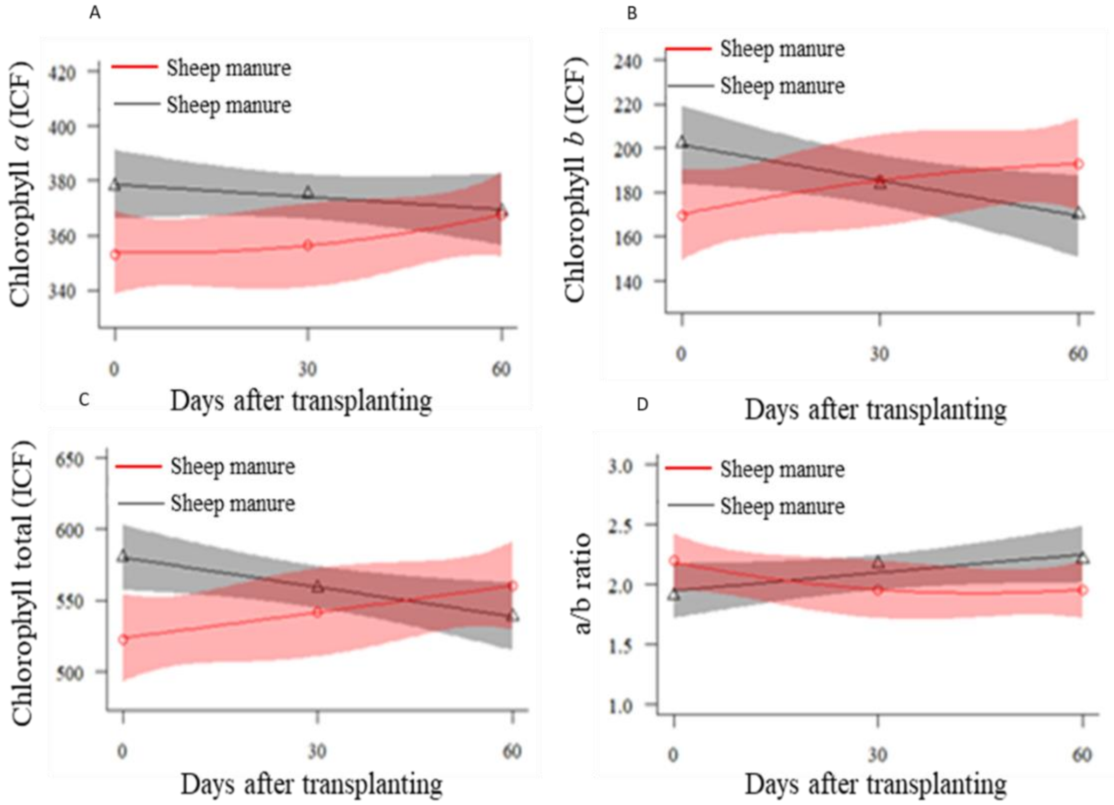

Figure 6. A – chlorophyll a of green pepper plants under biofertilization and application times (Sheep manure and Cattle manure); B – chlorophyll b of green pepper plants under biofertilization and application times (Sheep manure and Cattle manure); C – total chlorophyll of green pepper plants under biofertilization and application times (Sheep manure and Cattle manure); D – chlorophyll a/b ratio of green pepper plants under biofertilization and application times (Sheep manure and Cattle manure).

There was no difference between biological fertilizers for chlorophylls and chlorophyll *a*/*b* ratio (Figure 7A-D) within the doses. This fact may be associated with the satisfactory level of organic matter in the soil and with the levels of N and Mg^{2+} present in the biofertilizers composition. Since, N and Mg^{2+} are essential for the formation of the chlorophyll molecule, promoting no differences in its production under ideal supply conditions.

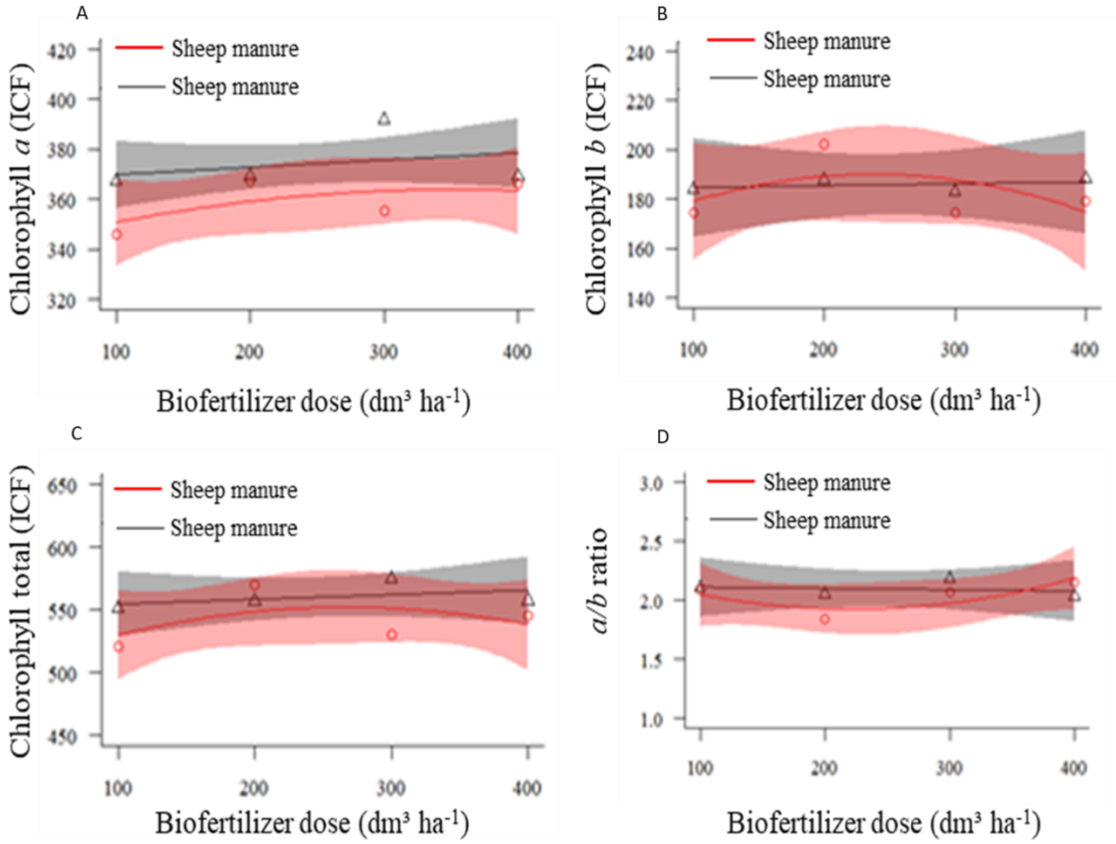

Figure 7. A – chlorophyll a of green pepper plants under biofertilization in different doses (Sheep manure and Cattle manure); B – chlorophyll b of green pepper plants under biofertilization in different doses (Sheep manure and Cattle manure); C – total chlorophyll of green pepper plants under biofertilization in different doses (Sheep manure and Cattle manure); D – chlorophyll a/b ratio of green pepper plants under biofertilization in different doses (Sheep manure and Cattle manure).

For some crops, there is a positive correlation between the chlorophyll concentration and the concentration of foliar N, since about 70% of foliar N is found in chloroplasts, which is essential for the synthesis and structure of chlorophyll molecules (Souza et al. 2020). Sousa et al. (2013) observed that the use of cattle biofertilizer increased the photosynthetic rate and chlorophyll (SPAD index) in *Jatrophacurcas* plants and showed that as the application of the biofertilizer increased, there was a linear increase in the chlorophyll content. However, this behavior was not observed in the present study.

4. Conclusions

The Hybrid green pepper cv Solário showed greater intrinsic efficiency of carboxylation at 60 DAT with the application of SBF, greater stomatal conductance, greater liquid photosynthesis, greater transpiration rate, and better water use efficiency, which results in a greater plant fresh weight at the time of flowering induction.

The concentration of internal carbon iC is higher at 30 DAT with the application of CBF.

The dose of 400 dm³ ha^{-1} of SBF provides higher values of stomatal conductance, and the doses of 200 and 300 dm³ ha^{-1} of CBF promotes a higher concentration of internal carbon.

SBF applied at 60 DAT promotes greater intrinsic carboxylation efficiency.

CBF promotes higher values of the total chlorophyll content when applied 0 DAT.

Authors' Contributions: LEAL, Y.H .: conception and design, acquisition of data, analysis and interpretation of date, drafting the article; DIAS, T.J.: conception and design, analysis and interpretation of data, drafting the article; SOUZA, A.G.: analysis and interpretation of data, drafting the article; BEZERRA, A.C.: drafting the article; RODRIGUES, L.S.: acquisition of data, analysis and interpretation of data; ALBUQUERQUE, M.B.: analysis and interpretation of data, drafting the article; LEAL, M.P.S.: acquisition of data, analysis and interpretation of data; SILVA, A.J.: acquisition of data, analysis and interpretation of data; LUCENA, M.F.R.: acquisition of data, analysis and interpretation of data. All authors have read and approved the final version of the manuscript.

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Ethics Approval: Not applicable.

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