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Gas exchanges in the zucchini culture fertilized with biofertilizers in two types of soil

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Abstract

The application of biofertilizers may provide plants similar performance and even superior to the use of mineral fertilizers, therefore, the determination of the most suitable sources to be applied in different crops is relevant. Given the importance of gas exchange in the performance of crops and that biofertilizers can provide increases in these physiological parameters, this study aimed to evaluate gas exchange in zucchini plants cultivated in two types of soil and managed with different aerobic fermentation biofertilizers. The experiment was carried out in full sunlight, in pots, using a randomized blocks design in an arrangement of split plots. The plots consisted of two types of soil (S_1: Red-Yellow Argisol and S_2: Fluvic Neossol), subdivided plots into five aerobic fermentation biofertilizers (F_1 = quail, F_2 = sheep, F_3 = mixed - bovine + quail + sheep, F_4 = bovine, and F_5 = enriched crab), with five replicates. At 45 days after transplanting, the following variables were analyzed: photosynthesis, stomatal conductance, transpiration, internal CO_2 concentration, leaf temperature, and chlorophyll. Bovine, sheep, and mixed biofertilizers showed the highest rates with the best performance of Italian zucchini cultivation. The types of soil studied concerning the rates did not have a significant effect between treatments on zucchini cultivation. Moreover, Chlorophyll was not significantly influenced by any of the treatments applied.

Keywords: *Cucurbita pepo*, organic fertilizers, photosynthesis

Introduction

Italian zucchini (*Cucurbita pepo* L.) has highlighted among the plants grown for human consumption due to its elevated trading potential, representing a productive option all year round to producers, with good acceptance from the consumer market (Azambuja et al., 2015).

This vegetable has been cultivated in all regions of Brazil, mainly through family farming, carried out by owners of small farms. In the present scenario, guided by the search for sustainable food production, which tries to expand the production per hectare, decreasing the environmental impacts, biofertilizers have been used as an alternative to achieve this aim by reducing production costs. It is worth mentioning that the use of mineral fertilizers increases the costs of food production. Thus, by producing biofertilizers, it contributes to the optimization of the organic residue utilization, mainly in family-based properties, minimizing environmental impacts, in addition to contributing to fertilization (Diniz et al., 2011; Sousa et al., 2013).

The manufacturing process of organic inputs can be made from aerobic fermentation, resulting from the mixture of one or more manure, that may or may not be enriched with other materials, and water (Viana et al., 2013). Biofertilizers are easy to manufacture; moreover, it can be own-produced by farmers, generating inputs economies (chemical fertilizers). Still, it promotes improvements in environmental sanitation, reducing the contamination of the groundwater, enabling the proper waste disposal, and even reducing the emission of greenhouse gases (Drumond et al., 2010).

It is important to adequately supply the nutrients that the plants need because the absence of essential plants nutrients might cause nutritional stress, which can anticipate the senescence of the leaves, prejudice the absorption of CO_2, causing the closure of stomata in

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order to decrease the perspiration and, consequently, affecting photosynthetic rates (Epstein & Bloom, 2006). When photosynthetic rates are negatively affected, plants may have the development reduced, which could cause irreversible effects on crop production parameters.

The beneficial effects of the application of organic inputs have been observed in studies conducted in different cultures of agronomic and economic interest. In the culture of *Jatropha curca* L. with the application of bovine biofertilizer and forage with bovine wastewater was observed reading values in the SPAD (Soil Plant Analysis Development), which demonstrated an increase in the chlorophyll values in the leaves of these plants (Sousa et al., 2013; Erthal et al., 2010). Similarly, increases in chlorophyll contents were observed in melon plants when crab biofertilizer was applied (Ferreira et al., 2011).

Based on the above, the present study aimed to evaluate gas exchange in zucchini plants grown in two types of soil and fertilized with different aerobic fermentation biofertilizers.

Material and Methods

The experiment was carried out from September to November 2017, in an experimental area at the Meteorological Station of the Federal University of Ceará, in Fortaleza, Ceará, located at the geographic coordinates 03°44'45'' South latitude, 38°34'55'' West longitude, at altitude of 19.5 m. The climate of the region, according to Köppen (1923), is Aw', characterized as a tropical rainy climate with predominant rainfall between the summer and autumn seasons. The monthly average data of the climatic variables gathered during the experiment are shown in Table 1.

Table 1. Average monthly data of climatic variables during the experiment period.

Month	Air Temperature (°C)	Relative Humidity (%)	Wind Speed (m s^{-1})	Precipitation (mm)
September	26.6	67	4.3	3.5
October	27.0	70	4.1	8.4
November	27.0	70	4.0	0.7

Source: Agrometeorological station – UFC.

There was no considerable variation in the average values of air temperature, relative humidity, and wind speed during the experimental period, it can be observed that there was higher rainfall in October (Table 1).

The experimental design used was in randomized blocks in split plots, with five replications. The plots consisted of two types of soil (S_1: Red-Yellow Argisol and S_2: Fluvic Neossol), subdivided plots into five aerobic fermentation biofertilizers (F_1 = quail, F_2 = sheep, F_3 = mixed - bovine + quail + sheep, F_4 = bovine, and F_5 = enriched crab), with five replicates. Each plot unit was formed by two pots, containing one plant per pot, totaling 100 plants.

The experiment was carried out in full sunlight, in pots with a capacity of 25 L, filled with two different soils, in which zucchini (*Cucurbita pepo* L.) seedlings, Corona F1 hybrid, were transplanted. The soils used as substrates were the Red-Yellow Argisol with a sandy clay loam texture and the Fluvic Neossol with a sandy loam texture (EMBRAPA, 2018). The chemical and physical characteristics of the 0 to 0.20 m layer are shown in Table 2.

As a way of supplementing the nutrient needs of the crop and based on the results of soil analysis, five types of aerobic fermentation biofertilizers prepared from different sources were used: quail, sheep, bovine manure, bovine mixture x quail x sheep and crab remain.

Plastic tanks with a volumetric capacity of 310 L were employed to prepare the biofertilizers. In the preparation of simple quail, sheep, and bovine biofertilizers, respectively, the ingredients used were 100 L of fresh manure and 100 L of water, in a 1:1 ratio, fermenting for 30 days (Viana et al., 2013). The same methodology was followed in the preparation of the mixed biofertilizer, modifying the amounts of manure used, 50 L of bovine manure, 25 L of quail manure and 25 L of sheep manure.

In the preparation of the enriched crab biofertilizer, it was necessary to mix 60 kg of ground crab stubble (leg and head), 5 kg of crushed brown sugar, 2.0 L of milk, and 100 L of water, leaving to ferment for one 90 days (Sousa et al., 2016). During the fermentation period, the biofertilizers were manually turned, daily, using a stick-type aerator, to ensure homogeneity and stimulate aeration.

After the fermentation period, a chemical analysis of the biofertilizers was performed; the characteristics are shown in Table 3. The analyzes were made using the methodologies suggested by Malavolta et al. (1997).

Table 2. Result of chemical and physical analysis of soils (layer 0 to 0.20 m) used as substrates in the experiment.

Chemical attributes	Argisol	Neosol
Ca^{2+} ($cmol_c\ kg^{-1}$)	1.20	3.70
Mg^{2+} ($cmol_c\ kg^{-1}$)	0.60	2.40
Na^{+} ($cmol_c\ kg^{-1}$)	0.23	0.03
K^{+} ($cmol_c\ kg^{-1}$)	0.36	0.37
$H^{++}\ Al^{3+}$ ($cmol_c\ kg^{-1}$)	1.98	1.32
Al^{3+} ($cmol_c\ kg^{-1}$)	0.15	0.10
SB ($cmol_c\ kg^{-1}$)	2.60	6.50
CCE ($cmol_c\ kg^{-1}$)	4.60	7.82
pH (H_2O 1:2.5)	6.00	6.40
CE ($dS\ m^{-1}$)	0.35	0.46
V (%)	57	83
m (%)	5	2
ESP (%)	5	1
C ($g\ kg^{-1}$)	6.48	6.96
N ($g\ kg^{-1}$)	0.61	0.76
$C\ N^{-1}$	11	9
OM ($g\ kg^{-1}$)	11.17	12.00
P ($g\ kg^{-1}$)	32	43
Physical Attributes	Argisol	Neosol
	Granulometric composition ($g\ kg^{-1}$)	
Sand	620	563
Silt	100	309
Clay	280	125
Textural Class	Light loam	Sandy loam
Soil Density ($g\ kg^{-1}$)	1.52	1.40

SB = sum of bases; CCE = capacity of cations exchange; EC = electrical conductivity; V = base saturation; m = percent of aluminum saturation; ESP = exchangeable sodium percentage; OM = organic matter.

Table 3. Composition of essential nutrients in the dry matter of aerobic fermentation biofertilizers originated from quail, sheep, mixed, bovine and crab tailings.

	Chemical characteristics				
	N	P	K	Ca	Mg
Biofertilizers	---------------------g L^{-1}---------------------				
Quail	3.90	0.33	2.50	1.50	0.60
Sheep	0.26	0.26	4.20	4.00	0.90
Mixed*	1.70	1.16	2.80	2.50	0.75
Bovine	0.82	1.40	1.00	2.50	0.75
Crab	0.30	1.10	2.30	3.20	0.30

Mixed* = bovine + quail +sheep.

Intending to meet the nutritional requirements of the plant, the maximum recommendation of chemical fertilization for the zucchini cultivation, according to Filgueira (2012) was adopted, corresponding to: 140 kg ha^{-1} of nitrogen (Urea - 45% N), 300 kg ha^{-1} of phosphorus (simple superphosphate - 18% P_2O_5) and 150 kg ha^{-1} of potassium (Potassium chloride - 60% K_2O). As a reference, for a stand of 16,667 plants per hectare (spacing of 1.0 x 0.6 m), the maximum recommended dosage per plant in the cycle was: 14 g of N; 30 g of P and 15 g of K. There was no application of limestone or micronutrients.

From the recommended values and chemical analysis of the soils, it was possible to determine the need for plant nutritional supplementation in the different types of soils. It was considered the substrate densities (1.52 and 1.40 g cm^{-3}), volume (25 L for both), matter (38 kg and 35 kg) for Argisol and Neossol, respectively (Table 4).

Table 4. Recommended amounts of nutrients, present in the substrates and nutritional supplementation needs.

Chemical characteristics	Nutrient		
	N	P	K
Recommendation		(g $plant^{-1}$)	
	14	30	15
Argisol		($g\ kg^{-1}$)	
	0.61	0.032	0.14
		(g 38 kg^{-1})*	
	23.18	1.22	5.32
Neosol		($g\ kg^{-1}$)	
	0.76	0.043	0.14
		(g 35 kg^{-1})*	
	26.6	1.51	4.9
Nutritional supplementation needs		(g $plant^{-1}$)	
Argisol	-	28.78	9.68
Neosol	-	28.49	10.1

* = values calculated by multiplying the density of the substrates (Argisol 1.52 g kg^{-1} and Neossol 1.40 g kg^{-1}) by the volume of the vases.

It was tried, in the treatment, to provide an adequate dosage, to meet the maximum fertilization recommendation for N, P, and K, with a maximum value of 9 L plant^{-1}, applied manually (Table 5).

Table 5. Amount of nutrients supplied from the application of biofertilizers to soils.

Biofertilizers	N	P	K
	g (9 applications)$^{-1}$		
Quail	35.1	2.97	22.5
Sheep	2.34	2.34	37.8
Mixed	15.3	1.44	25.2
Bovine	7.38	12.6	9.0
Crab	2.7	9.9	20.7

Applied dose = 1 L plant^{-1} week^{-1}.

The sprinkler irrigation used in the experiment was a self-compensating drip type with a flow of 4 L h^{-1}; the irrigation blade applied daily was quantified from the evaporation measured in a class "A" tank.

During maximum vegetative growth of the culture, at 45 days after transplantation (DAT), measurements of gas exchange were performed in all treatments. The readings were taken in the morning (between 09:00 and 11:00 h). An infrared gas analyzer (IRGA, mod. LCi System, ADC, Hoddesdon, UK) was used to measures the variables, in an open system, with an airflow of 300 mL min^{-1}. The following parameters were measured: photosynthesis, stomatal conductance, transpiration, internal carbon dioxide concentration, and leaf temperature; measurements were made on the first fully expanded leaf from the apex, using an artificial radiation source (about 1200 μmol m^{-2} s^{-1}).

For the SPAD (Soil Plant Analysis Development) or IRC (Chlorophyll Relative Index) indexes, also at 45 DAT, assessments of the nutritional status of zucchini plants were carried out in all treatments, measured with a chlorophyll meter (Chlorophyll Meter SPAD-502 Minolta Co., Japan). The measurements were made on the same leaves on which the gas exchange was determined using the methodology described by Oliveira et al. (2013).

The data of the evaluated variables were submitted to analysis of variance by the F test, at the level of 1 and 5% probability, and the means were compared to the Tukey test, using the software ASSISTAT 7.6 beta (Silva & Azevedo, 2016), and Microsoft Office Excel (2010).

Results and Discussion

The summary of the analysis of variance for photosynthesis (A), stomatal conductance (gs), transpiration (E), internal CO_2 concentration (C_I), leaf temperature (T_L) and SPAD index in zucchini plants depending on the type of soil and the applied biofertilizers are shown in Table 6.

Table 6. Summary of the analysis of variance for photosynthesis (A), stomatal conductance (gs), transpiration (E), internal CO_2 concentration (C_I), leaf temperature (T_L) and SPAD index in zucchini plants depending on the type of soil and the applied biofertilizers.

SV	DF	A μmol m^{-2} s^{-1}	gs mol m^{-2} s^{-1}	E mmol m^{-2} s^{-1}	C_I mmol m^{-2} s^{-1}	T_L °C	SPAD
Soils - A	1	0.91^{ns}	0.04^{ns}	0.03^{ns}	0.73^{ns}	0.07^{ns}	4.96^{ns}
Residue - a	6	20.25	0.007	5.01	4561.16	5.61	101.85
Plots	7	-	-	-	-	-	-
Biofertilizers - B	4	9.53**	14.93**	15.82**	2.22^{ns}	0.49^{ns}	2.62^{ns}
Residue - b	24	21.61	0.008	2.77	4769.7	0.13	35.96
Iteraction - A x B	4	1.64^{ns}	0.63^{ns}	0.8^{ns}	0.7^{ns}	0.22^{ns}	1.15^{ns}
Total	39	-	-	-	-	-	-
CV% - a	-	30.79	31.81	35.24	23.57	7.0	21.54
CV% - b	-	31.8	33.1	26.22	24.1	1.07	12.8

SV = source of variation; DF = degrees of freedom; CV = coefficient of variation; ^{ns} = non-significant; * = significant at 5% probability; ** = significant at 1% probability.

It is possible to notice that the treatments applied did not have significant interaction in any of the evaluated parameters; although, there was only a significant isolated effect for the types of biofertilizer applied in the values of photosynthesis, stomatal conductance, and transpiration, at the significance levels of 1% (p <0, 01) (Table 6). It is also observed that regarding the variables internal CO_2 concentration, leaf temperature, and SPAD index, there was no relevant effect of any treatments applied, different soils, and biofertilizers (Table 6).

The absence of a significant difference between treatments concerning the internal CO_2 concentration and leaf temperature (Table 6) was also observed by Gomes et al. (2015) when applying bovine biofertilizer on sunflower plants, using Red-Yellow Argisol as substrate. Likewise, Sousa et al. (2016) when applying crab biofertilizer to corn plants, they found no significant effects for the leaf temperature variable.

The significant effect of the application of the different biofertilizers on the mean values of the photosynthetic rates (A) of zucchini plants, measured at 45 DAT, can be seen in Figure 1. Moreover, plants that received the ovine (17.9 µmol m^{-2} s^{-1}), bovine (19.4 µmol m^{-2} s^{-1}) and mixed (17.5 µmol m^{-2} s^{-1}) biofertilizers had higher photosynthetic rates than the plants that were fertilized with quail (9.7 µmol m^{-2} s^{-1}) and crab (8.6 µmol m^{-2} s^{-1}) biofertilizers (Figure 1).

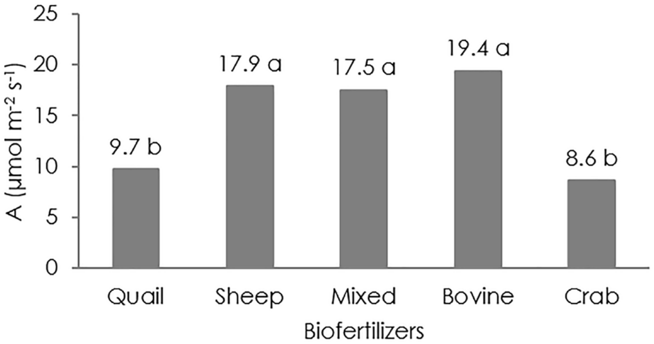

Figure 1. Zucchini photosynthesis rate (A) as a function of the types of biofertilizers applied.

The higher values in bovine, sheep, and mixed biofertilizers stood out because, besides presenting a nutritional wealth, they also showed higher levels of organic matter, which may have contributed to higher photosynthetic rates (Figure 1). Larcher (2006), states that the highest photosynthetic rates are achieved through adequate fertilization.

Dantas et al. (2020) in cultivation of zucchini plants, applying bovine biofertilizer, reported trends similar to that of this study at 45 days after sowing in a Neolithic Regolitic soil. These authors obtained values of photosynthesis about 17, 16, and 15 µ mol m2 s^{-1} at concentrations of 19, 20, and 28% of biofertilizer. Furthermore, Viana et al. (2013), when investigating the effect of biofertilizers (simple and mixed) on melon plants, that belong to the same family as the zucchini plant, reported a positive influence of these organic inputs at 60 DAT, with values nearby to those presented in this study. These authors did the work under the same conditions of the present study.

Regarding the stomatal conductance (gs) of the zucchini plants evaluated, the significant effect of the application of the different biofertilizers, measured at 45 DAT (Figure 2).

It can be seen that the stomatal conductance rate was statistically higher when applied to bovine (0.40 mol m^{-2} s^{-1}), ovine (0.38 mol m^{-2} s^{-1}), and mixed (0.27 mol m^{-2} s^{-1}) biofertilizers. The lowest rates were noticed on quail (0.14 mol m^{-2} s^{-1}) and crab (0.15 mol m^{-2} s^{-1}) biofertilizers (Figure 2). It should be noted that through these treatments, higher levels of organic matter were obtained (quail (7.86 g Kg^{-1}), sheep (8.79 g Kg^{-1}), mixed (12.31 g Kg^{-1}), bovine (14.79 g Kg^{-1}), crab (8.17 g Kg^{-1})), providing higher moisture to the soil.

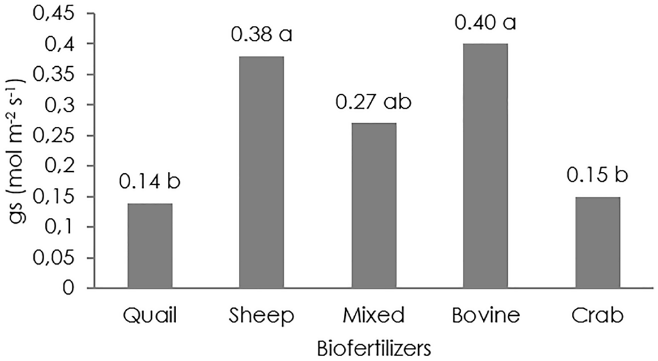

Figure 2. Zucchini stomatal conductance rate (gs) as a function of the types of biofertilizers applied.

Under these conditions (Figure 2), the plant has better performance and, consequently, longer stoma opening time to capture atmospheric CO_2 (VIANA et al., 2014). Similarly, in a study conducted by Viana et al. (2013) investigating the effect of biofertilizers (simple and mixed) on melon plant leaves, found a positive effect concerning the stomatal conductance rate, obtaining values that corroborate the results given in this research. Dantas et al. (2020), who observed maximum values of stomatal conductance varying between 0.32 and 0.2 mol m^{-2} s^{-1} in zucchini plants, under different concentrations of bovine biofertilizer.

On the other hand, Sousa et al. (2013) did not find a significant effect of the application of bovine biofertilizer regarding stomatal conductance in physic nut plants at 51 days after transplanting. The bovine biofertilizer used by these authors was produced using manure collected from the same stable of the present study, following the same methodology. It is most likely the presence of biofertilizers, sheep, bovine and mixed, allowed better content of nutrients available to plants in the soil, increasing the nutrition of this crop. Resulting, therefore, in higher values of stomatal conductance in the leaves of plants that received these treatments. According to Taiz et al. (2017), the insufficient supply of nutrients to plants may cause physiological disturbances, induce reductions in photosynthetic rates, and even impair their development.

The significant effect of the application of different biofertilizers on the mean transpiration values (E) of zucchini plants evaluated, measured at 45 DAT, are shown in Figure 3.

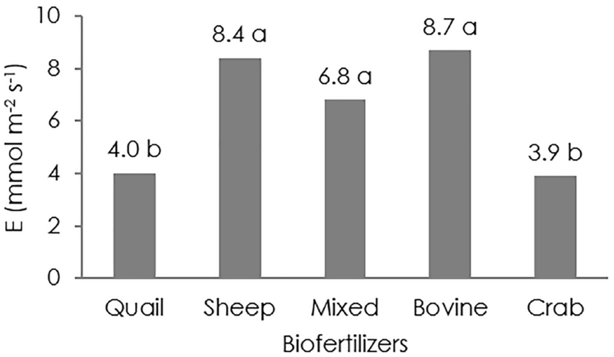

Figure 3. Zucchini transpiration rate (E) as a function of the types of biofertilizers applied.

It can be seen that the transpiration rate was statistically superior when applied to bovine (8.7 mmol m^{-2} s^{-1}), ovine (8.4 mmol m^{-2} s^{-1}), and mixed (6.8 mmol m^{-2} s^{-1}) biofertilizers compared to quail (4.0 mmol m^{-2} s^{-1}) and crab (3.9 mmol m^{-2} s^{-1}) biofertilizers (Figure 3).

These biofertilizers, differently from quail and crab biofertilizers, may have provided an adequate amount of nutrients and soil moisture, favoring gas exchange in plants (Figure 3).

Studying plants that belong to the same family of zucchini plants, some authors evaluated biofertilization effects on plants' transpiration. Sousa et al. (2020), studying okra plants, observed that the biofertilizer provided the maximum value of transpiration in the evaluated plants. Viana et al. (2013), studying melon plants fertilized with different types and doses of biofertilizers, observed that the bovine biofertilizer provided better transpiration values than the mixed biofertilizer. These authors attribute the results obtained to the different salinity of the biofertilizers used.

Moreover, the improvement in the values of photosynthesis, stomatal conductance, and transpiration provided by sheep, mixed and bovine biofertilizers concerning quail and crab biofertilizers indicate that the number of nutrients present in the first biofertilizers was more effective than the present in the others. It can also be assumed that this superiority was provided by the most suitable soil moisture.

According to Silva et al. (2010), depending on the amount of water in the soil available to plants, the stomatal closure may occur, which will limit stomatal conductance and transpiration, thus reducing photosynthesis. Although quail and crab biofertilizers have improved soil moisture, they may have caused some toxicity to plants, which has led to the most unfavorable results.

Conclusions

Bovine, ovine, and mixed biofertilizers enable the zucchini plants to have higher rates of photosynthesis, stomatal conductance, and transpiration, which consequently allows the best performance of this culture.

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