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Correlation of physiological and productive parameters of cowpea under organic fertilization[1]

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ABSTRACT - The work was intended to evaluate the effect of different organic compounds originating from the mixture of agroindustrial and urban wastes, as well as their doses in the physiology and production of cowpea. The experiment was conducted under a randomized block design, in a 4 x 5 + 2 factorial arrangement, with four organic fertilizers (3 composed obtained from of sewage sludge, green coconut shell, carnauba butt; and sewage sludge biochar), five doses (20, 40, 60, 80 and 100 t ha^{-1}) and two additional treatments (without fertilization and mineral fertilization). We evaluated the leaf gas exchange in three phenological stages and the productive aspects. Regarding the physiological variables, the compounds with carnauba butt and green coconut shell promoted the best results and throughout the crop cycle, the gas exchange decreased. The productive aspects were increased by about 60%, especially due to the treatments with organic compounds. The PCA can confirm the univariate results, with emphasis on compounds with green coconut shell and carnauba butt. The high percentage of explanation found in axis 1 (60.49%) may be associated with the fact that the gas exchange and production variables are highly responsive to fertilization. The treatment with biochar caused a reduction in both in the physiological and in the productive aspects. The compounds with carnauba tip and green coconut shell promote satisfactory results, being viable alternatives to complement chemical fertilization with an indicated dose of 300g pot^{-1} or 60 t ha^{-1}.

Key words: Principal component analysis. Waste destination. Plant physiology. Production. *Vigna unguiculata*.

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INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is a crop originally from the African continent that has great socioeconomic importance, especially because its seeds are made up of numerous favorable nutritional characteristics, being an important source of proteins, energy, fibers and minerals, making this legume one of the most cultivated and consumed around the world (FREIRE FILHO *et al*., 2007; MELO *et al*., 2017). In Brazil, cowpea is a highlight in the semi-arid region, where this crop is responsible for generating employment and income for numerous families (PAIVA *et al*., 2018).

The crop has great adaptability to different edaphoclimatic conditions, but some factors, such as water deficit, fertilization and, consequently, the availability of nutrients, can affect growth and physiological responses, which directly reflects in the productivity of the plants (COSTA *et al*., 2020; SILVA *et al*., 2021). Among the main physiological changes related to the low availability of nutrients, we can mention the reduction of stomatal conductance and photosynthesis. According Dourado Neto *et al*. (2012), fertilization is a decisive factor in plant development, however, when used in a disorderly manner, chemical fertilization can cause irreversible environmental impacts.

In view of the environmental problems caused by the excessive and indiscriminate use of chemical fertilizers, alternatives aimed to improve the production chain in a more sustainable way, through the use of organic compounds and biosolid wastes, are being studied (COSTA *et al*., 2017). The organic materials most commonly used by farmers are organic compounds, which are fertilizers from the composting process, which can promote improvements in the chemical, physical and biological properties of the soil (BRANDANI *et al*., 2019; LARNEY; ANGERS, 2012).

Among the agroindustrial wastes, we can mention the carnauba butt and the green coconut shell as environmental liabilities with great utility and good physical and chemical characteristics, which can be used as raw materials for composting. Another waste that has been extensively studied for application in agriculture, due to the expansion of basic sanitation and the search for an adequate destination, is sewage sludge, a semi-solid waste, rich in some macro and micronutrients (LOPES *et al*., 2018).

According to Callegari and Capodaglio (2018), before being used in any agricultural activity, sewage sludge must be treated to reduce or eliminate pathogens and the potential for putrefaction, where pyrolysis is one of the ways to stabilize this waste for the production of biochar. When applied in adequate doses, biochar helps in the development of plants, thus contributing to reduce the emission of greenhouse gases, improve soil microbiota and increase water absorption (MADARI; MAIA; NOVOTNY, 2012; REZENDE *et al*., 2016).

Even with the importance of cowpea and the problems caused by the excessive application of chemical fertilizers, studies that relate the effect of organic fertilizers on the physiological and productive responses of this crop and a correlation of univariate and multivariate data are still necessary. According to Füzy *et al*. (2019), multivariate analysis is used to interpret the results more comprehensively, and, among the multivariate methods, we have Principal Component Analysis (PCA), a widely used technique to obtain a set of non-orthogonal correlated axes.

In this context, this work was intended to evaluate the effect of different organic compounds originating from the mixture of agroindustrial and urban wastes, as well as their doses in the physiology and production of cowpea using univariate and multivariate analysis.

MATERIAL AND METHODS

The experiment was conducted from April to June 2019, in a greenhouse covered with 200 microns UV plastic, located in Fortaleza - Ceará, with latitude of 3°43’02” S and longitude of 38° 32’35” W. During the experimental period, the maximum temperature ranged from 28.7 to 29.6 ºC, while the minimum ranged from 16.7 to 16.9 ºC, according to the data obtained through a datalogger installed in the greenhouse.

The experimental design used was in randomized blocks, in a 4 x 5 + 2 factorial arrangement, with four organic fertilizers (3 composed obtained from of sewage sludge, green coconut shell, carnauba butt; and sewage sludge biochar), five doses (100, 200, 300, 400 and 500 g pot^{-1}) corresponding to (20, 40, 60, 80 and 100 t ha^{-1}), two additional treatments (without fertilization and mineral fertilization) and five repetitions, totaling 110 experimental units. We used cowpea seeds and BRS-Pujante cultivars, which sown in plastic pots with a capacity of 12 dm³, containing soil classified as Red-Yellow Argisol, collected in the Experimental Field de Pacajus, belonging to Tropical Embrapa Agroindustry.

The chemical fertilizer, used in the additional treatment, was applied to the soil, 30 days before sowing, according to the methodology of Faquin, Vale and Furtini Neto (2008), providing, in each vessel, 12 g of triple superphosphate, 2.5 g of magnesium sulphate, 4 g of potassium chloride, 8 g of calcium nitrate, 123 mg of zinc sulphate, 83 mg of manganese sulphate, 33 mg of copper sulphate and 29 mg of boric acid.

The organic compounds were obtained from sewage sludge, green coconut shell and carnauba butt, mixed with chicken manure, in the proportion of 3:1 (v/v) + plaster, arranged under ridges in rows with 2.5 m long, 2 m wide and 1 m high. The ridges were turned over weekly for a period of seven months in order to assist in the composting process. The sewage sludge biochar was obtained through the pyrolysis process oven with a heating rate of 10 ºC min^{-1}, at a final carbonization temperature between 400 and 500 ºC and absence of oxygen.

Later, the organic compounds were crushed, sieved in a 5 mm mesh, placed to dry for 48 hours in an oven with forced air circulation at 45 ºC and, subsequently, weighed according to the treatments: 100, 200, 300, 400 and 500 g mixed with soil (13 kg pot^{-1}) + 1.25 g of limestone per pot, with the aid of an electronic mixer, for a period of 10 minutes. After the incubation period (30 days) of the treatments, soil samples were collected with the aid of an auger, at a depth of 20 cm; and, after natural drying, they were subjected to routine chemical analysis, micronutrients and electrical conductivity, and the results are shown in Table 1.

Irrigation was determined by the gravimetric method, maintaining the crop at 80% of the water retention capacity (WRC). During the cultivation cycle, the necessary cultural treatments were also carried out for the proper conduction of the crop, such as staking and elimination of weeds.

At 20, 30 and 50 days after sowing - DAS (vegetative, pre-flowering and reproductive period), we carried out measurements of the liquid photosynthetic rate (*A*), stomatal conductance (g_s), transpiration rate (*E*) and internal concentration of CO_2 (Ci) in fully developed leaves, between 9:00 am and 12:00 pm, using an infrared gas analyzer (IRGA; LCI BioScientfic model). With these data in hand, the instantaneous water use efficiency (WUE: *A/E*) and the instantaneous carboxylation efficiency (ICE) were calculated (*A*/Ci).

Table 1 - Chemical properties of the soil after application of treatments

Trat.	Dose	MO	P	Zn	Mn	Fe	Cu	K	Mg	Ca	H+Al	pH	EC
	(g pot^{-1})	(g.dm^3)	--------------- (mg.dm^3) ---------------					----------- (mmol$_c$.dm^3) -----------				-	(mS.cm)
S + CB	100	5.4	79	2.4	3.7	10	0.2	0.7	4	30.8	3.3	6.7	2.85
	200	5.8	186	3.9	6.6	12	0.2	0.8	5.3	47.5	4.1	6.9	3.16
	300	5.9	265	6.3	9.2	13	0.3	0.9	7	66.9	2.3	6.9	3.54
	400	6.7	302	7.2	11.1	15	0.35	1	7.8	73.6	0	7.3	3.36
	500	7	308	8.3	13.6	16	0.35	1.3	9.7	91.1	0	7.3	3.51
S + CC	100	4.8	92	2.6	5.1	9	0.25	0.5	4.9	33.4	0	7.2	2.79
	200	5.1	236	4.6	6.7	11	0.35	0.7	6.2	49.2	0	7.3	2.91
	300	6.4	276	7.1	10.6	13	0.35	0.8	7.7	64.6	0	7.3	3.23
	400	6.6	304	8	12.6	14	0.35	1	8.1	69.4	0	7.4	3.29
	500	6.8	281	9.6	12.9	14	0.35	1.2	10	81.1	0	7.4	3.3
S + CS	100	5.7	64	15.9	5.4	27	4.65	0.7	4	19.1	6.1	6.3	2.21
	200	6.4	151	27	7.4	42	9.25	1	4.2	29.1	5.3	6.3	2.69
	300	6.6	193	38.8	9.5	46	12.7	1.2	4.9	35	8.7	6.2	3.06
	400	7.5	243	50.8	12	59	17.5	1.2	4.8	36.9	6.4	6.3	3.14
	500	10.3	303	59.8	15.5	70	21.7	1.5	6	47.6	9.1	6.4	3.5
S + SB	100	6	57	21.6	4.5	49	3.95	0.5	5.6	12.3	0	7	2.61
	200	6.5	104	41	8	88	8.05	0.6	7.8	18.2	5	6.9	3.26
	300	9.5	160	58	11.3	129	12.55	0.7	10.2	24.5	5.6	6.8	4.13
	400	10.7	208	71.1	14.4	154	15.25	0.7	11.6	30	3.3	6.8	4.56
	500	10.9	245	77.5	15.6	157	16.5	0.7	12.1	30.7	4.8	6.9	4.69
C	-	4.5	154	1.9	6.5	21	0.85	1.9	5.6	13.4	15	5.5	3.31
WF	-	3.4	5	0.5	2.6	5	1	0.3	3.3	6.4	9.8	6.4	1.12

Treatments: S + CB (soil + compound with carnauba butt), S + CC (soil + compost with crushed coconut), S + CS (soil + compound with sewage sludge), S + SB (soil + sludge biochar), C (chemical) and WF (without fertilization). EC = Electric conductivity

With regard to the production variables, from the beginning of the reproductive phase (60 DAS), weekly collections of the mature pods were carried out, and then they were placed in an oven at 45 ºC to complete the drying process; subsequently, the analyses were made in order to obtain data regarding the pods length (PL), number of pods (NP), pods weight (PW), number of seeds per pod (NSP) and production (PROD).

The qualitative data of the analyzed variables were subjected to variance analysis and when significant by the F test, subjected to the Tukey means test with $p < 0.05$. For quantitative data, regression analysis was performed, the equations that best fit the data were selected based on the significance of the regression coefficients at $p < 0.01$ and $p > 0.05$ probability by the F test. For the statistical analysis and preparation of the charts, the Assistat 7.6 Beta (SILVA; AZEVEDO, 2016) and Sigmaplot (version 11.0) computer programs were used, respectively. The physiological variables at 50 DAS and production were also analyzed using the multivariate statistical method named Principal Component Analysis (PCA), with the aid of the R statistical package.

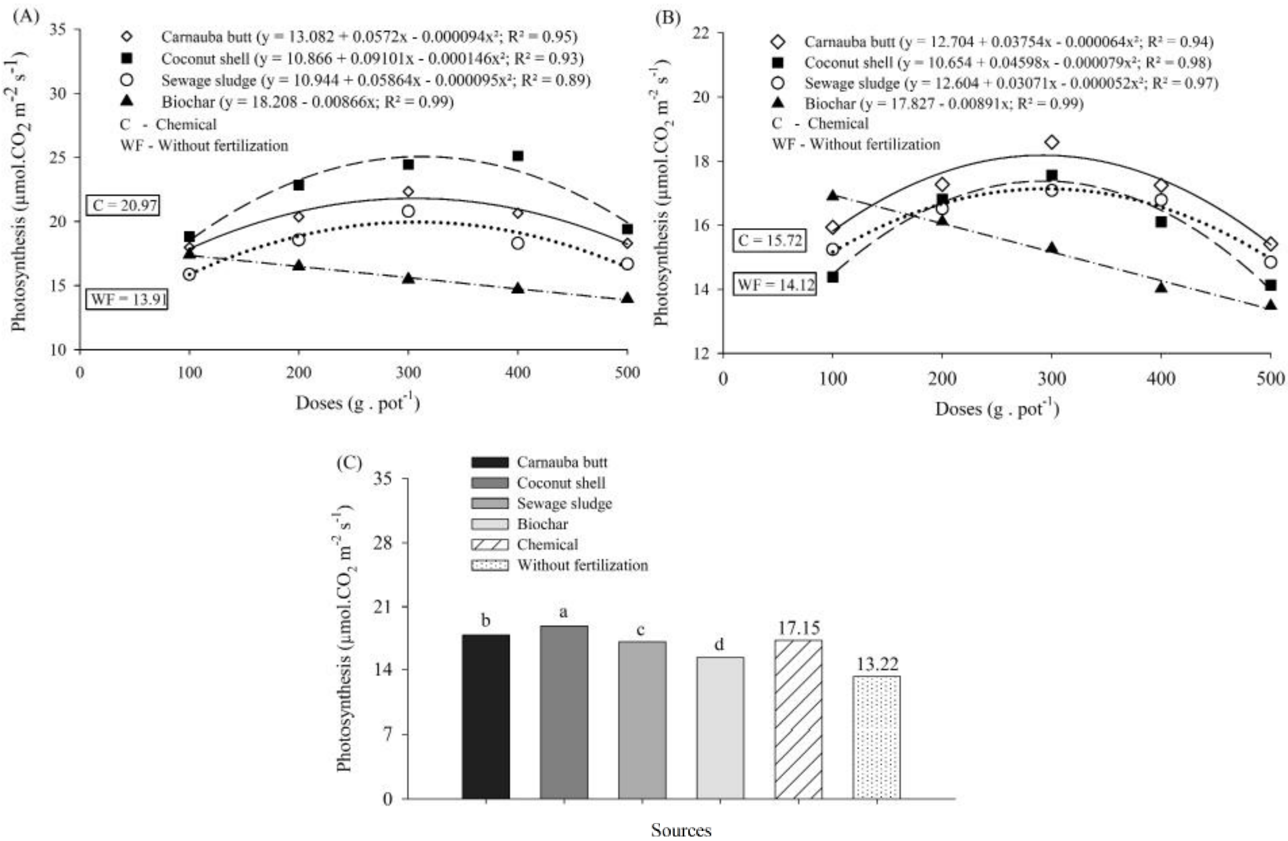
RESULTS AND DISCUSSION

Gas exchange

Gas exchange was significantly influenced by the treatments tested. For photosynthesis in the vegetative period (Figure 1 A), we found that the green coconut shell compound showed the best results, for all tested doses, followed by the compound with carnauba butt (CB), finding the maximum points of 25.05 and 21.78 µmol CO_2 m^{-2} s^{-1}, respectively. For the treatments with biochar, we found a reduction of 19.95%. In turn, in the pre-flowering period (Figure 1 B), we found that the organic compound of CB was the one that showed the best results. The results in the reproductive period (50 DAS) indicate that the organic compound of green coconut shell was significantly superior to the others (Figure 1 C).

The light and the nutrition of the plants exert a great influence on the physiological responses and on the production, since they are extremely important factors for the adequate performance of the photosynthetic apparatus (TAIZ *et al*., 2017). Accordingly, the results for the photosynthetic rate can be substantiated by this

Figure 1 - Photosynthesis in cowpea plants at 20 (A), 30 (B) and 50 (C) days after sowing, grown under different fertilizer sources and doses



**Significance at 1% probability

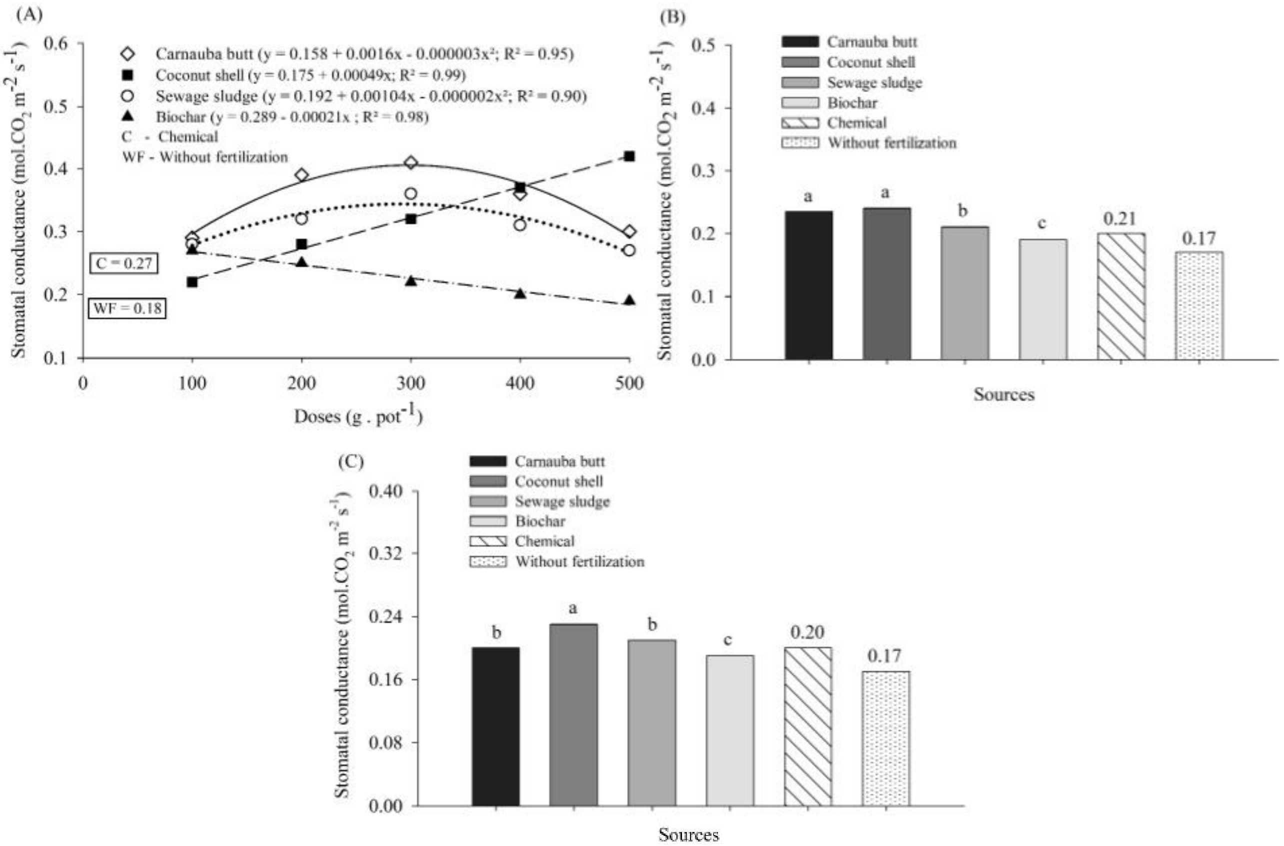
hypothesis, since, in the vegetative and pre-flowering period, the plants responded positively up to a certain dose, with the exception of treatments with biochar, possibly as a response to the excess of Zn and Cu (Table 1).

It is worth underlining that the increase in photosynthesis noted in the vegetative and pre-flowering periods can be explained by the larger leaf area found in the referred stages, as this is a factor that influences the interception of radiation and also due to the reproductive stage already approaching the end of the cycle in which the plant reduces its photosynthetic capacity. This reduction in the growth rate of the crop, after flowering, is due to the translocation of photoassimilates, which culminates in the reduction of the leaf area index and explains the decline in the photosynthetic rate in the reproductive stage (TEIXEIRA; STONE; HEINEMANN, 2015).

Regarding stomatal conductance during the vegetative period (Figure 2 A), we found the maximum points of 0.37 and 0.33 μmol CO_2 m^{-2} s^{-1} for the compounds of carnauba butt and sewage sludge, respectively. For compound of green coconut shell, we noted an increasing linear trend, with an increase of 0.00049 μmol CO_2 m^{-2} s^{-1} for each increase of 1g pot^{-1}, while, for biochar, there was an opposite response, with a decrease of 0.00021 μmol CO_2 m^{-2} s^{-1}. For the pre-flowering (Figure 2 B) and reproductive (Figure 2 C) stages, we noted similar responses for g_s, where the compound of green coconut shell was significantly superior, with the exception of the treatment with carnauba butt in the pre-flowering.

The potassium is one of the nutrients responsible for stomatal opening and closing (MARSCHNER, 2012), its availability and assimilation represent important factors in g_s. In this sense, Prazeres *et al.* (2015), when working with two cowpea cultivars and potassium doses, found a steady increase in g_s as a function of potassium doses. Thus, the superior results found when the bean plants were fertilized with compounds of carnauba butt, green coconut shell and sewage sludge, in both stages of development, are related to the higher amount of K in the soil in relation to the treatments with biochar (Table 1). In addition, the reduction of g_s can cause decreased photosynthetic activity, which can be easily noted in the results.

Figure 2 - Stomatal conductance in cowpea plants at 20 (A), 30 (B) and 50 (C) days after sowing, grown under different fertilizer sources and doses



**Significance at 1% probability

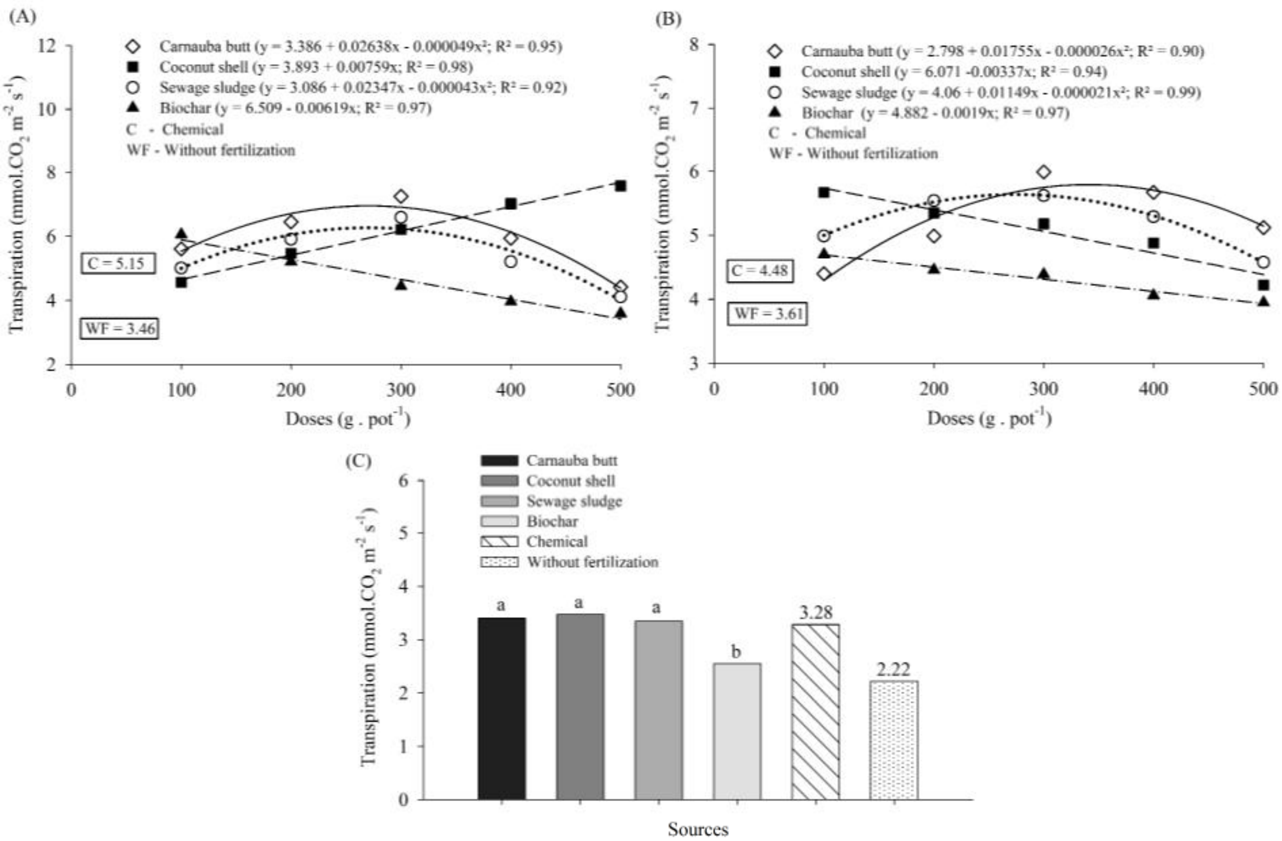
With regard to the variable on transpiration in the vegetative period (Figure 3 A), we found a maximum point of 6.94 µmol CO_2 m^{-2} s^{-1} for the compound of carnauba butt, while the compound of green coconut shell presented a linear increase, reaching the highest dose with an average of 7.69 µmol CO_2 m^{-2} s^{-1}. For the pre-flowering period (Figure 3 B), treatments with coconut shell and biochar showed a linear reduction of 0.0033 and 0.00196 µmol CO_2 m^{-2} s^{-1}, respectively, and treatments with carnauba butt and sewage sludge showed maximum points of 5.76 and 5.63 µmol CO_2 m^{-2} s^{-1}, respectively. In the reproductive period (Figure 3 C), we noted that the organic compounds presented averages superior to the biochar.

Knowing that transpiration is directly related to stomatal opening and the influx of atmospheric CO_2 into the leaf, we can relate the positive results of variables *A*, g_s and *E* with an adequate supply of water and nutrients to the plants. It is worth underlining that the reduction in *E* is more noticeable at the reproductive stage, since the plant initiates the senescence process and its energy expenditure is metabolized especially for the production of pods. In line, Oliveira *et al*. (2018), when evaluating the physiological responses of cherry tomatoes at different phenological stages, found a significant reduction in transpiration in the reproductive period.

For instantaneous carboxylation efficiency (*EiC; A/Ci*), we found similar responses in the vegetative and in pre-flowering (Figure 4 A and B), as in these stages, the compound of green coconut shell promoted an increase of 0.00011 and 0.00004, respectively, while biochar caused a linear reduction of 0.00003 and 0.00004, respectively. The *EiC* data when the plants were fertilized with carnauba butt and sewage sludge compounds, in the vegetative period (Figure 4 A), showed maximum points of 0.076 and 0.073, while, in the pre-flowering period, the compost of carnauba butt showed a maximum point of 0.06 (Figure 4 B). In the reproductive period (Figure 4 C), linear reductions were noted for sewage sludge and biochar.

The probable explanation for the increase in *EiC* in plants fertilized with the compound of green coconut shell

Figure 3 - Transpiration in cowpea plants at 20 (A), 30 (B) and 50 (C) days after sowing, grown under different fertilizer sources and doses



**Significance at 1% probability

and carnauba butt, in comparison with the other treatments, may be related, mainly, to the adequate supplementation of phosphorus that acts as a structural element of nucleotides and phospholipids, as well as energy transfer and activation of some enzymes in the Calvin-Benson cycle (LI *et al*., 2016).

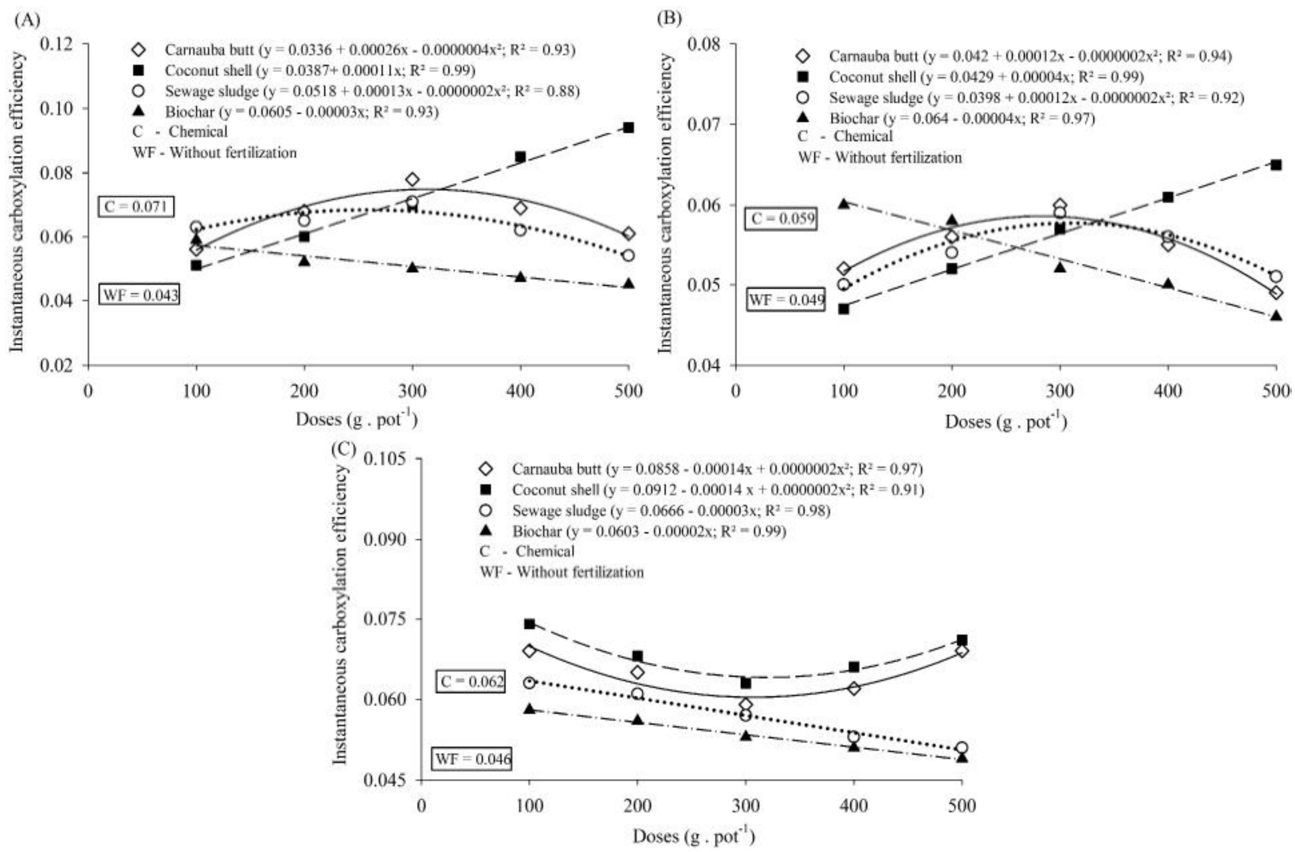
For to the instantaneous efficiency of water use (*EUA*; *A/E*), in the vegetative period, we found the minimum points of 3.34 and 3.11 μmol CO_2 m^{-2} s^{-1} H_2O for compounds with green coconut shell and sewage sludge, respectively. For the treatments with carnauba butt and sewage sludge biochar there were linear increments (Figure 5 A). For the pre-flowering stage (Figure 5 B), for carnauba butt and sewage sludge, finding maximum points of 3.52 and 3.19 μmol CO_2 m^{-2} s^{-1} H_2O. For the treatments with biochar, we noted a linear reduction of 10.41%, while, for the compound of coconut shell, there was a linear increase of 26.71% (Figure 5 B).

Water is the most relevant environmental factor for the development of plants and is of paramount importance for the productive success. It is worth underlining that the increase in *EUA* can be associated with the reduction of stomatal conductance, since the plant closes its stomata to reduce the loss of water to the atmosphere. Thus, the positive results found in the vegetative period (20 DAS) for treatment with biochar can be explained by the reduction of g_s.

Productive variables

As for the productive variables, only that pod length (PL) and number of seeds per pod (NSP) were not significant. Chemical fertilization provided the highest number of pods, in relation to the other treatments (Figure 6 A). Regarding the pods weight (Figure 6 B), we found the maximum points of 45.95 g for the treatments with sewage sludge and 41.74 g for the green coconut shell (CS). The treatments with carnauba butt promoted a linear increase, while the biochar caused a total linear reduction of 47.65%. For the production (Figure 6 C), were finding the maximum points of 35.21 and 33.44 g $plant^{-1}$ for the treatments with sewage sludge and CS.

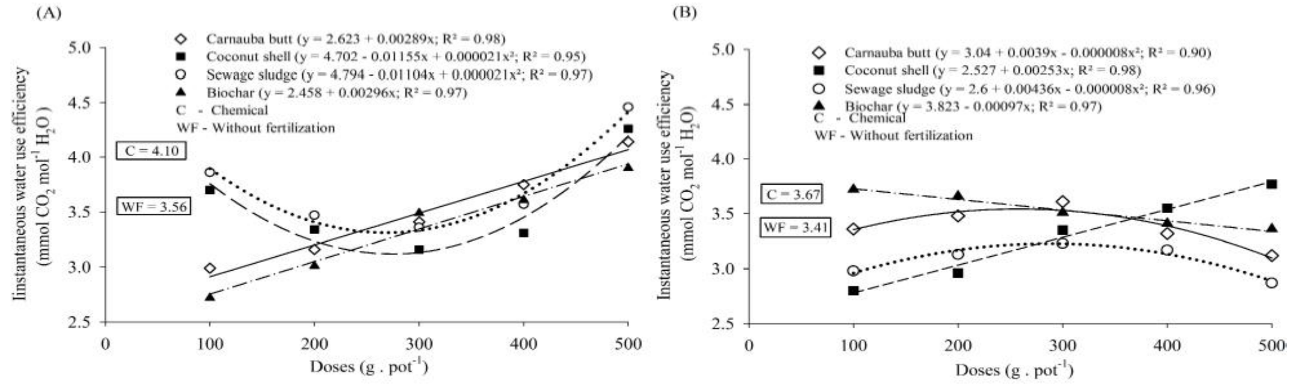
Figure 4 - Instantaneous carboxylation efficiency in cowpea plants at 20 (A), 30 (B) and 50 (C) days after sowing, grown under different fertilizer sources and doses



**Significance at 1% probability

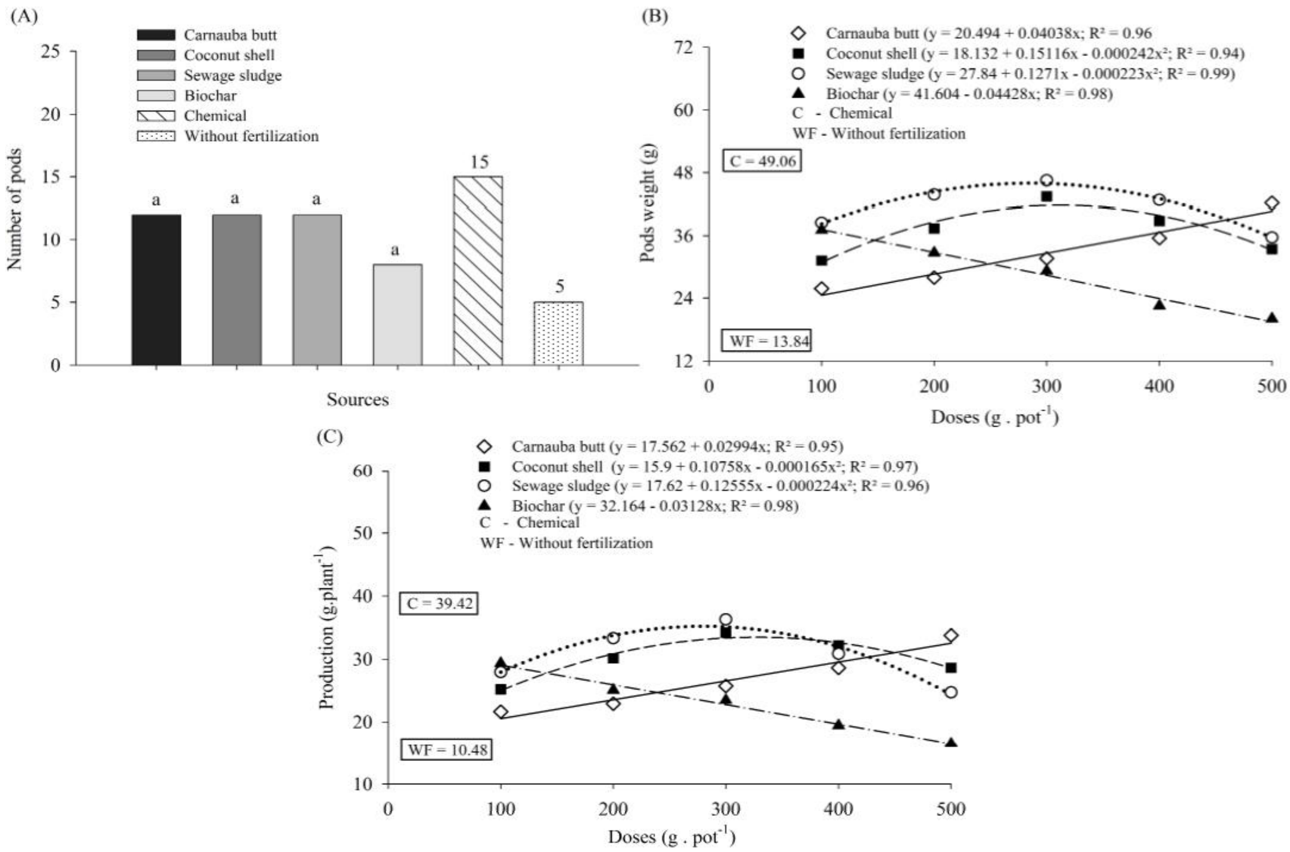
Our results corroborate with of Lobo, Grassi Filho and Büll (2012), who evaluated the effect of nitrogen and sewage sludge on beans production, when they found that the number of pods and grain yield were higher when the plants were fertilized with compound of sewage sludge, especially associating these results with nitrogen. Therefore, the positive results found for the productive aspects when the plants were fertilized with sewage sludge

Figure 5 - Instantaneous water use efficiency in cowpea plants at 20 (A) and 30 (B) days after sowing, grown under different fertilizer sources and doses



**; *Significance at 5% and 1% probability, respectively

Figure 6 - Number of pods (A), pods weight (B) and production (C) of cowpea plants, grown under different fertilizer sources and doses



**Significance at 1% probability

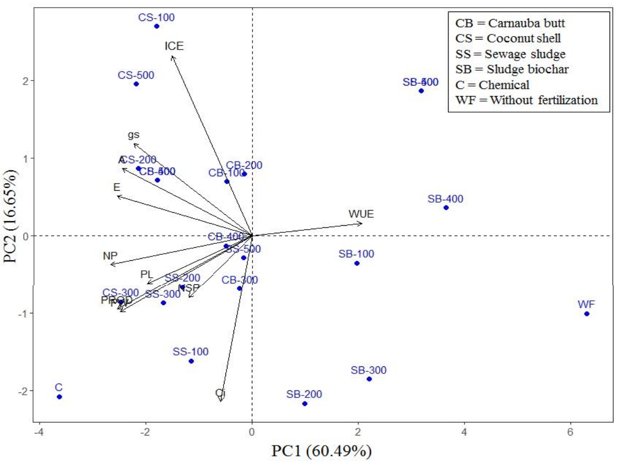
are probably due to the supply of satisfactory amounts of nitrogen and potassium (Table 1), once that N and K are some of the nutrients most demanded by beans, being determinant in grain yield (MOREIRA *et al*., 2013).

Principal component analysis

With the data of gas exchange in the reproductive period and the productive parameters, we carried out an principal component analysis (PCA), where two of the principal components accounted for 77.14% of the total variance (Figure 7). The PCA revealed the effects of organic fertilizers on the physiological and productive responses, where the physiological variables, except the EUA, showed a greater correlation with the compounds of green coconut shell and carnauba butt. As for the production variables, all had a high correlation with chemical fertilization and with the compound of green coconut shell at a dose of 300 g pot^{-1} or 60 t ha^{-1}. All variables were negatively correlated with biochar and with treatment without fertilization.

Accordingly, it is noticeable that the PCA can confirm the univariate results, showing once again the emphasis on compounds with green coconut shell and carnauba butt (Figure 7). The high percentage of explanation found in axis 1 (60.49%) may be associated with the fact that the gas exchange and production variables are highly responsive to fertilization. Similarly, Campelo *et al*. (2019), when evaluating the physiological parameters related to water stress in forest species, using a multivariate approach, associated the high percentages of explanation of the axes with the correlation of the variables with the tested treatments.

Figure 7 - Principal component analysis of gas exchange (*A*, g_s, *E*, ICE and WUE) at 50 days after sowing and of the productive aspects (PL, NP, PW, NSP and PROD) in cowpea plants grown under different fertilizer sources and doses



CONCLUSIONS

1. The organic compounds with agroindustrial wastes of carnauba butt and green coconut shell are to be viable alternatives for partial replacement or complementation to chemical fertilization, because they improved the physiological and productive aspects of cowpea, and the indicated dose is 300 g pot^{-1} or 60 t ha^{-1};
2. The PCA confirm the univariate results, showing once again the emphasis on compounds with green coconut shell and carnauba butt;
3. The biochar of sewage sludge promotes a reduction in physiological and productive parameters, indicating a toxic effect on plants. Therefore, it is necessary to carry out experiments with doses lower than the lowest dose tested in new research.

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