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Growth and formation of bean phytomass (*Vigna unguiculata* L.) fertilized with mineral fertilizer and *manipueira*

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Abstract

The alarming increase of utilization of chemical fertilizers for crops has stimulated the quest for organic residues with the potential for enhancing agricultural products. The *manipueira* is a liquid residue produced in the process of making flour or starch from cassava (*Manihot esculenta* Crantz.) with potential for use as agricultural fertilizers because of its rich macronutrient (K, N, Mg, P, Ca and S, in the quantitative order) content and with sufficient contents of all micronutrients except molybdenum. Thus, the objective of this work was to evaluate the initial development of cowpea bean (*Vigna unguiculata* (L.) Walp., cv. Corujinha) fertilized with mineral fertilization and *manipueira*. The research was performed between January and February 2017, in a greenhouse located in the city of Campina Grande (7º 13'50" S, 35º 52'52" W, 551 m asl), state of Paraíba, Brazil. The experimental design was completely randomized, involving 8 treatments and 3 replicates, totally 24 experimental units. The treatments included applying mineral fertilization using phosphorus; potassium; phosphorus and potassium in combination; organo-mineral fertilization with phosphorus and *manipueira*; and phosphorus combined with *manipueira* in different proportions (25, 50, 75 and 100 % of the *manipueira* recommended doses). Stem diameter, plant height, number of leaves, fresh shoot biomass and dry biomass of the aerial parts of the plant, fresh root biomass and dry root biomass were determined at 15 days after sowing. Data were subjected to the analysis of variance by F-test and the averages were compared by Tukey's test ($p < 0.05$). The results showed significant differences between the treatments evaluated. The most significant increases in bean growth and phytomass accumulation were occurred upon mineral fertilization with phosphorus on stem diameter, plant height, fresh shoot biomass and fresh and dry root biomass. Plants subjected to fertilization with phosphorus and different doses of *manipueira* exhibited a negative effect on the initial development stage of the cowpea (V4 stage) and it is not recommended as a substitute for the mineral fertilization for this culture in this period.

Keywords: agroindustrial waste; biofertilizer; cassava wastewater; chemical fertilizer; leguminous.

Abbreviations: CRD_completely randomized experimental design; CV_coefficient of variation; DAS_days after sowing; EC_{se}_electrical conductivity of soil saturation extract; DB_dry biomass of the aerial part of the plant (stem and leaves); DF_degrees of freedom; DRB_dry root biomass; EC_w_electrical conductivity of water; FRB_fresh root biomass; FSB_fresh shoot biomass of the aerial part of the plant (stem and leaves); NL_number of leaves; PET_polyethylene terephthalate; SD_stem diameter; T_treatment.

Introduction

Bean is a legume of great importance in the diet of human. Besides being an excellent source of proteins, it also has essential amino acids, carbohydrates, vitamins, minerals, dietary fibers and low amounts of fat. It is extensively cultivated in the arid and semi-arid parts of Brazil and it forms a significant portion of the diet of these regions. This culture is still important for family farming and it contributes to the generation of jobs and income, both in rural and urban areas (Lima et al., 2007; Lima et al., 2011). Nearly 70% of the beans grown in the Brazilian territory are common bean varieties (*Phaseolus vulgaris*) and 30 % of cowpea (*Vigna unguiculata* (L.) Walp). However, in the Northeast states and Northern parts, cowpea variety is the most widely grown (Coutinho et al., 2014).

According to Laing et al. (1984), the biological cycle of the bean is divided into vegetative and reproductive phases. The vegetative phase consists of the stages: V0 – germination (beginning of seed germination), V1 – emergence (50 % of the cotyledons out of the soil), V2 – primary leaves (pair of expanded primary leaves), V3 – first trifoliate leaf (with expanded leaflets) and V4 – third trifoliate leaf (with expanded leaflets). The reproductive phase consists of the stages: R5 – pre-flowering (after floral button issuing or inflorescence), R6 – flowering (first open flower), R7 – vegetable formation (first pod with the corolla detached), R8

– filling of vegetables (beginning of pod swelling), R9 – maturation (when the first pod starts to discolor or dry).

Although beans are tropical crops adapted to poorly fertile soils, sowing the low productive traditional cultivars and the use of low technology has resulted in reduced grain yields. On the other side, cowpea yields can reach even 3000 kg ha^{-1} under favorable conditions of soil fertility (Galvão et al., 2013).

Limited provision of phosphorus (P) during the initial bean phenological stage may restrict the plant growth and; therefore, adversely influence the final yield (Grant et al., 2001). Potassium (K) such as P is another element which is one of the nutrients that most affects the yield in the tropical soils. It is highly mobile in the soil, being its involvement in the processes of photosynthesis, translocation and ionic balance (Galvão et al., 2013). The cowpea; however, is not exigent in nitrogen fertilization because of the nodulation capacity and efficient nitrogen fixation system by the plant. Therefore, recommendations for the fertilization of the bean crop must be done based on the soil chemical analyzes for the phosphorus and potassium contents (Andrade Jr. et al., 2002).

In order to increase the cowpea yield, high doses of chemical fertilizer are being intensely utilized. In light of this, applying organic residues to the crop appears to be a substitute due to their ability to minimize the mineral fertilizer doses added to the soil. Crop management employing agro-industrial residues has been appreciated as a sustainable alternative from the social, environmental and economic standpoints, since these residues have large amounts of nutrients that can increase agricultural yield through improvement in soil fertility, when incorporated into soil (Modesto et al., 2009; Dantas et al., 2016). This strategy is mainly indicated for small farms, where the financial and technological resources are scarce, taking advantage of agricultural by-products that are often discarded (Medeiros and Lopes, 2006).

Of the various agro-industrial residues showing potential for use as agricultural fertilizers, there is the *manipueira*. *Manipueira* is a residue produced in the process of making flour or starch from cassava (*Manihot esculenta* Crantz). It contains high organic load and toxic cyanogenic glycosides (linamarin) that can cause environmental risks in the case of inappropriate disposal in the environment. However, *manipueira* is rich in macronutrients such as: nitrogen (1386.0 ppm), phosphorus (283.0 ppm), potassium (2600.0 ppm), and calcium (210.0 ppm). The *manipueira* also contains micronutrients like magnesium, copper, zinc and manganese (Leonel; Cereda, 1995; Cardoso et al., 2009; Conceição et al., 2013; Nasu et al., 2015). However, before it can be safety utilized as biofertilizer, treatments involving a minimum of 15 days of anaerobic fermentation in a hermetic system is required to volatilize the toxic and polluting cyanide acid before its loading to the soil and plant (Oliveira et al., 2013).

Authors such as Marini and Marinho (2011), Dantas et al. (2017) and Magalhães et al. (2015) evaluated the use of *manipueira* as a source of nutrients in agricultural crops. However, there are few comparative studies between *manipueira* as organic fertilizer and chemical fertilizers, involving cowpea bean.

Thus, the objective of this work was to evaluate the initial development of cowpea bean (*Vigna unguiculata* (L.) Walp), cv. Corujinha, fertilized with mineral fertilization and *manipueira*.

Results and discussion

Effect of fertilization on growth variables

The effect of the variable 'plant height' analyzed by the Tukey's test, was significant at 1 % of probability and presented 9.22 % of coefficient of variation (CV) for the different treatments at 15 DAS. The mineral fertilization treatment resulted in taller plants compared with the other treatments utilizing *manipueira* in different doses. A similar finding was noted for stem diameter, in which a statistical significant difference ($p < 0.01$) and low CV (8.27 %) were reported based on the classification of Gomes (1985). The largest diameter was obtained for mineral fertilization with phosphorus, followed by fertilization with phosphorus and potassium. No significant statistical difference for number of leaves was reported between the treatments, with a CV of 23.72 %. However, the mineral fertilization treatment with phosphorus and potassium produced the highest average (5.5 leaves per plant). The variance analysis and the recorded averages are shown at Tables 1 and 2, respectively.

The current findings in this study concur with those of Schwengber et al. (2010) in their work on cowpea. They reported that the use of *manipueira* reduced the plant growth; however, plants treated with mineral fertilization showed better development than those fertilized with this residue. Magalhães et al. (2014) in their research on maize, reported similar results, in which the plant heights and stem diameters decreased with increasing doses of *manipueira*.

The high potassium content of *manipueira* (above 2000 mg L^{-1}) exceeds that of the residues like sugarcane vinasse, the final by-product of sugarcane (Fioretto, 1994). The excess potassium ion may be toxic to the plants because of the antagonism that could adversely affect the absorption of other essential elements necessary for plant development, principally during the initial phase of plant growth (Malavolta, 1997).

Effect of fertilization on phytomass accumulation

The analysis of variance also revealed significant statistical differences ($p < 0.01$) in the fresh shoot biomass and dry biomass of the aerial part of the plant (FSB and DB, respectively), and fresh root biomass (FRB) and dry root biomass (DRB) for the different types of fertilization performed in the study (Table 3).

The results of Tukey's test ($p < 0.05$) showed that the greatest increase in the fresh shoot biomass (stem and leaves) occurred for the plants treated with mineral fertilization: P_2O_5 (10.95 g), P_2O_5 + KCl (7.67 g) and KCl (4.97 g). However, the combination of mineral fertilization and *manipueira* (P_2O_5 + *manipueira*) provided an average value of fresh shoot biomass equal to 4.46 g, being similar to that obtained by treatment with KCl. The other treatments involving mineral fertilizations in combination with increasing doses of *manipueira* (P_2O_5 + recommended dose of *manipueira* + 25, 50, 75 and 100 % of the *manipueira* recommendation) produced the least fresh shoot biomass values, without statistically differentiating between them (Fig. 1).

Table 1. Analysis of variance for plant height (PH), stem diameter (SD) and number of leaves (NL) at 15 days after sowing (15 DAS).

Source of variation	DF	Average squares		
		PH	SD	NL
Treatments	7	42.499524**	0.4413333**	2.617570 ^{ns}
Error	16	2.074167	0.095283	0.947917
Total corrected	23			
CV (%)		9.22	8.27	23.72

**, ns - significant at 1 % probability level ($p < 0.01$) and not significant at 5 % probability level ($p < 0.05$) by the Tukey's test, respectively; DF - degrees of freedom.

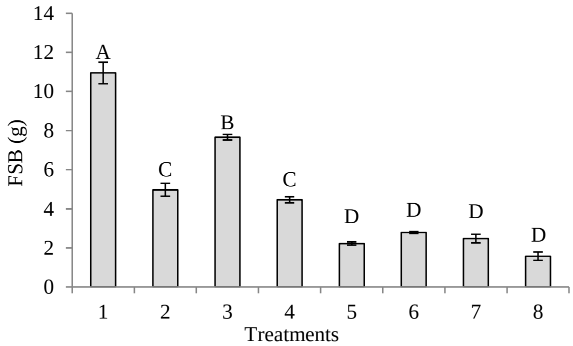

Fig 1. Fresh shoot biomass (FSB) in response to the fertilizers treatments applied. The same letters indicates no statistical significant difference between the treatments by the Tukey's test ($p < 0.01$). The treatments correspond to: (1) P_2O_5; (2) KCl; (3) P_2O_5 + KCl; (4) P_2O_5 + recommendation of *manipueira*; (5), (6), (7) and (8) P_2O_5 + recommendation of *manipueira* + 25, 50, 75 and 100 % of the *manipueira* recommendation, respectively.

Table 2. Observed average values of plant height (PH), stem diameter (SD) and number of leaves (NL).

Source of variation	Observed average values		
	PH (cm)	SD (mm)	NL
T1	23.80 a	4.48 a	5.00 a
T2	16.05 bc	3.60 b	4.50 a
T3	17.95 b	4.14 ab	5.50 a
T4	14.05 bc	3.49 b	4.00 a
T5	14.50 bc	3.67 ab	3.00 a
T6	12.73 c	3.34 b	4.50 a
T7	12.75 c	3.48 b	3.33 a
T8	13.10 c	3.66 ab	3.00 a

In each column, averages followed by the same letter show no statistical difference from each other by the Tukey's test at the 1 % probability level ($p < 0.01$). The treatments correspond to: (T1) P_2O_5; (T2) KCl; (T3) P_2O_5 + KCl; (T4) P_2O_5 + recommendation of *manipueira*; (T5), (T6), (T7) and (T8) P_2O_5 + recommendations of *manipueira* + 25, 50, 75 and 100 % of the *manipueira* recommendations, respectively.

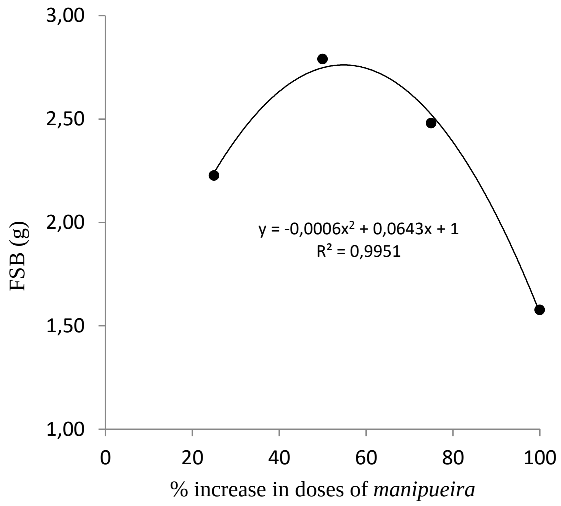

Fig 2. Effect of organo-mineral fertilization (P_2O_5 + recommendation of *manipueira* + 25, 50, 75 and 100 % of the *manipueira* recommendation) on fresh shoot biomass (FSB).

Table 3. Results of the analysis of variance for fresh shoot biomass and dry biomass of the aerial part of the plant (FSB and DB, respectively) and fresh and dry root biomass (FRB and DRB, respectively) as a function of the treatments applied.

Source of variation	DF	Average squares			
		FSB	DB¹	FRB	DRB
Treatments	7	31.034743**	0.132852**	0.043302**	0.002267**
Error	16	0.281088	0.007875	0.001879	0.000142
Total corrected	23				
CV (%)		11.43	13.40	9.62	17.42

** - significant at 1 % probability level ($p < 0.01$) by the Tukey's test; 1 - data transformed by √x.

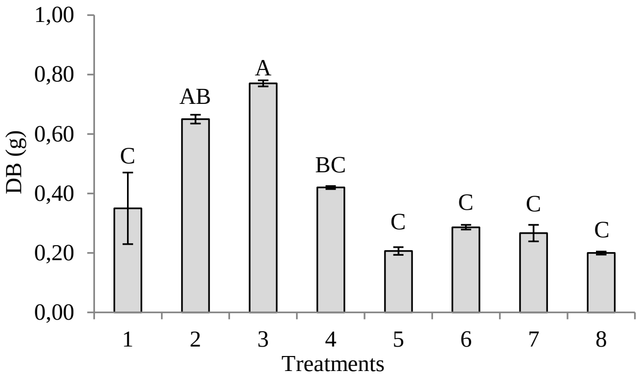

Fig 3. Dry biomass (DB) in response the mineral and organo-mineral treatments applied. The same letters indicates no statistical significant difference between the treatments by the Tukey's test ($p < 0.01$), where the treatments correspond to: (1) P_2O_5; (2) KCl; (3) P_2O_5 + KCl; (4) P_2O_5 + recommendation of *manipueira*; (5), (6), (7) and (8) P_2O_5 + recommendation of *manipueira* + 25, 50, 75 and 100 % of the *manipueira* recommendation, respectively.

Table 4. Results of the chemical characterization of the *manipueira*.

Parameters						
NTK	$N\text{-}NH_3$	$P\text{-}PO_4^{-3}$	K	Na	pH	CE
......................................g L^{-1}......................................					-	mS cm^{-1}
1,680	0,933	0,338	3,948	0,138	5,32	11,75

NTK: Total Kjeldahl Nitrogen; $N\text{-}NH_3$: Ammoniacal Nitrogen; $P\text{-}PO4^{-3}$: Soluble orthophosphate; K: Potassium; Na: Sodium; PH: Hydrogenionic potential and EC: Electrical conductivity.

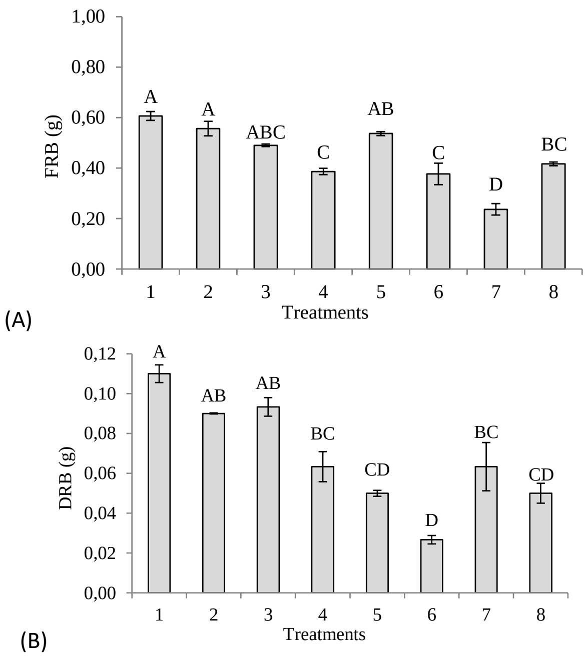

Fig 4. (A) Fresh root biomass (FRB) and (B) dry root biomass (DRB) as a function of the mineral and organo-mineral treatments applied. The same letters indicates no statistical significant difference between the treatments by the Tukey's test ($p < 0.01$), where the treatments correspond to: (1) P_2O_5; (2) KCl; (3) P_2O_5 + KCl; (4) P_2O_5 + recommendation of *manipueira*; (5), (6), (7) and (8) P_2O_5 + recommendation of *manipueira* + 25, 50, 75 and 100 % of the *manipueira* recommendation, respectively.

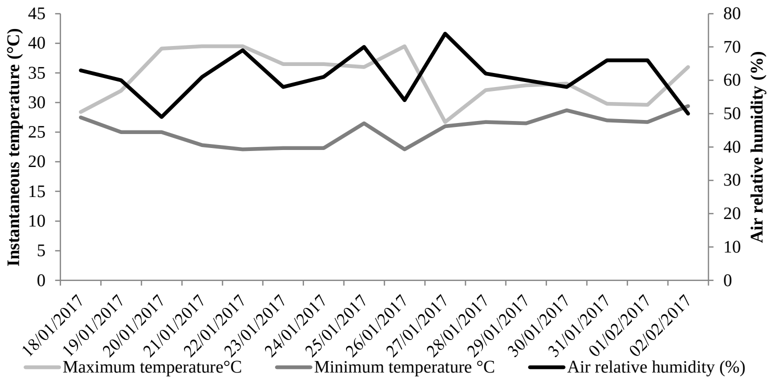

Fig 5. Air temperatures (°C) and air relative humidity (%) inside the greenhouse.

A quadratic effect was seen from the average values of fresh shoot biomass compared to the treatments involving mineral fertilization in combination with the increasing *manipueira* doses (25, 50, 75 and 100 %) (Fig. 2). The increased *manipueira* doses affected the cowpea crop, as evident in the decay of the fresh shoot biomass. The inflection point occurred at the 50 % dose apart from the recommended *manipueira* dosage. The coefficient of determination (R^2) seen from the graph adjustment was 0.99. In their work on subjecting lettuce cv. Regina 2000 to *manipueira* dosing, Duarte et al. (2012) confirmed a quadratic graph for leaf area similar to the present study. These authors reported that the increased *manipueira* doses increased the leaf area to the maximum, after which any heightened dosage only resulted in a reduction in the leaf area.

With reference to the dry biomass of the aerial part of the plant (DB), the phosphorus and *manipueira* treatments in different doses produced the lowest values for dry biomass differed significantly ($p < 0.01$) from sole mineral fertilizations (Fig. 3). The coefficients of variation for the variables of FSB and DB were 11.43 % and 13.40 %, respectively.

With respect to the fresh root biomass (FRB) and dry root biomass (DRB) (Figs. 4A and 4B, respectively), the highest averages were also linked to mineral fertilization; however, the plants growth under different concentrations of *manipueira* produced uneven results for both the variables mentioned. Barreto et al. (2014) showed that in the soils containing clayey fractions the nutrient availability and mobility as well as the soil water content are affected, similar to the soil used in the current study. This promotes a decline in the nutrient diffusion between the soil and the plant roots.

The present study revealed findings very different from the reports of Fonseca et al. (2016). In their work on soybean in pots of 4.5 L, the fresh mass of the root system showed a 28.11 % rise in response to the application of a single 100 mL dose of *manipueira*. They showed that the *manipueira* positively affected the development of this legume. Contrary to the current work, the *manipueira* doses failed to promote the growth in the variables assessed. The lowest *manipueira* dose used in this study (436.32 mL) applied to 10 L pots. It became clear that in proportion to the pot volume, this dose was nearly 50 % higher than the one employed by Fonseca et al. (2016). Thus, the *manipueira* doses used in the cowpea crop by organo-mineral fertilization may have been too high and, possibly, damaging to the crop.

Santos et al. (2010) verified an increase in the fresh and dry phytomass in their assessment on the response of lettuce to the addition of *manipueira* doses. However, from the maximum response points of the crop, there was a significant reduction of the values for these variables. The authors suggest that a potassium surplus may have occurred in the plant itself or in the soil, which obstructed the absorption of other nutrients by the plant roots.

The present work showed that the mineral fertilization using phosphorus resulted in a greater increase in plant height, stem diameter, fresh shoot biomass (stem and leaves), as well as fresh and dry root biomass of the cowpea bean. Potassium also positively affected the development variables of cowpea. However, applications that combined both chemical fertilizers (P_2O_5) and *manipueira* were deleterious to the crop in the initial developmental stage (V1, V2, V3 and V4 stages).

Materials and methods

Localization, experimental procedure and plant material

The experiments was performed in a greenhouse located in *Campus* I at Federal University of Campina Grande (UFCG), Campina Grande city (7º 13'50" S, 35º 52'52" W, 551 als), state of Paraíba, Brazil. Based on the Köppen classification adapted to Brazil (Coelho and Soncin, 1982), this area has the Csa type of climate, mesothermic, sub-humid, with a dry and hot dry season (4 to 5 months) and a rainy season extending from autumn to winter.

The 640 m² greenhouse with 2.5 m high had steel structure of the arch cover type. It was covered with low density 150 µm polyethylene, with the sides being covered with a shading screen having 80 % index protection. The maximum and minimum air temperatures and relative air humidity throughout the experimental period were measured using a digital thermo-hydrograph (Fig. 5). The research was conducted in 10 L polyethylene pots with 0.50 m spacing between the rows and 0.50 m between successive plants. To convert the pots into lysimeters of drainage, a hole was made in each pot at the bottom. A hose, with 15 cm in length and 6 mm in diameter, was connected to a 2 L plastic tank (PET bottles) that had been installed. In each pot a 0.04 kg layer of brita number 0 was placed, and covered with textile fabric and 12 kg of soil. This soil was taken from Esperança city, Paraíba and smashed and sieved through a 4 mm mesh sieve. A physico-chemical analysis of the soil was done through the UFCG Irrigation and Salinity Laboratory (LIS) and the results were as follows: pH in water (1: 2: 5) = 5.63; EC_{se} (electrical conductivity of soil saturation extract) = 0.61 mmhos cm^{-1}; Aluminum = 0.00 cmolc dm^{-3}; Magnesium = 2.99 cmolc dm^{-3}; Calcium = 3.49 cmolc dm^{-3}; Potassium = 0.21 cmolc dm^{-3}; Sodium = 0.17 cmolc dm^{-3}; Assimilable phosphorus = 1.82 cmolc dm^{-3}; Sulfur = 6.86 cmolc dm^{-3}; Organic carbon = 1.06 %; Organic matter = 1.83 %; Soil density = 1.13 g cm^{-3}; Textural classification: clay-loam soil. Once the pots were filled with the soil, the treatments were applied. They were then placed in the field capacity utilizing rainwater with electrical conductivity (EC_w) of 0.40 dS m^{-1} and, following which the sowing was performed. Cowpea cv. Corujinha, was sown on 01/18/17, by directly placing five seeds into each of the pots. After germination, three plants were maintained per pot. For the evaluations, the plant which presented average values of height and number of leaves was selected among the three. After 15 days from sowing (DAS) the experiment was finalized on 02/02/17.

Experimental design and treatments

The completely randomized experimental design (CRD) including eight treatments (T1, T2, T3, T4, T5, T6, T7 and T8) with three replications, amounting to a total of 24 experimental units was used. The treatments included mineral fertilization doses with phosphorus and potassium, as well as organic fertilization with *manipueira*, performed as follows: T1 - mineral fertilization was done using simple superphosphate (P_2O_5) as a phosphorus source; T2 - mineral fertilization with potassium chloride (KCl) as a potassium source; T3 - mineral fertilization with P_2O_5 and KCl as phosphorus and potassium sources, respectively; T4 - mineral fertilization with P_2O_5 as a phosphorus source and organic fertilization with *manipueira* having potassium as the reference macronutrient; T5 - involved mineral fertilization with P_2O_5 and organic fertilization with *manipueira* plus 25 % of the *manipueira's* recommended dose based on the reference macronutrient; T6 - included mineral fertilization with P_2O_5 and organic fertilization with *manipueira* plus 50 % of the *manipueira's* dose; T7 - utilized mineral fertilization with P_2O_5 and organic fertilization with *manipueira* plus 75 % of the *manipueira's* dose; T8 - included mineral fertilization with P_2O_5 and organic fertilization using *manipueira* plus 100 % of the *manipueira's* dose.

The *manipueira* doses were determined depending on the potassium content in this residue. The mineral and organo-mineral (phosphorus plus *manipueira*) fertilizations were assessed based on the recommendations of Novais et al. (1991) for experiments conducted in pots. Individual applications of the foundation were done in each pot as follows: 17.74 g of P_2O_5 (T1); 2.8 g of KCl (T2); 17.74 g of P_2O_5 and 2.80 g of KCl (T3); 17.74 g of P_2O_5 and 436.32 mL of *manipueira* (T4); 17.74 g of P_2O_5 and 436.32 mL of *manipueira* plus 109.08 mL of *manipueira*, corresponding to 25 % of the *manipueira* dosage (T5); 17.74 g of P_2O_5 and 436.32 mL of *manipueira* plus 218.16 mL of *manipueira*, corresponding to 50 % of the *manipueira* dosage (T6); 17.74 g of P_2O_5 and 436.32 mL of *manipueira* plus 327.24 mL of *manipueira*, corresponding to 75 % of the *manipueira* dosage; and finally, 17.74 g of P_2O_5 and 436.32 mL of *manipueira* plus 436.32 mL of *manipueira*, corresponding to 100 % of the *manipueira* dosage (T8). Before utilizing it with a nutrient source, the *manipueira* was placed in storage in an 85 L polyethylene container. Maintaining 5 cm of free surface in the polyethylene container, a hose with one end attached to the lid was immersed in a vessel containing water as an outlet for the gases generated during the effluent bio-digestion. After 60 days of storing the residue, chemical characterization was conducted based on the methodology prescribed in the Standard Methods for Wastewater (APHA, 2005) for use in the future as fertilizer. The *manipueira* was characterized by the Laboratory of Irrigation and Drainage Engineering of the UFCG and the findings given in Table 4.

Irrigation management

Irrigation was done daily using a graduated container with the aim of returning the soil to field capacity. The water volume added to each vessel was determined as a function of the difference between the volume applied on the prior day and the volume drained, in an attempt to restore to the soil the volume drained for nutrient cycling in the soil-plant system. The volume drained was completed with rainwater when irrigation was to be done (EC_w = 0.40 dS m^{-1}).

Traits measured

The variables of growth and phytomass accumulation were evaluated at 15 days after sowing (DAS) involving the vegetative phase of cowpea bean (V1, V2, V3 and V4 stages): plant height (PH), stem diameter (SD), number of leaves (NL), fresh shoot biomass (FSB), fresh root biomass (FRB), dry biomass (DB) and dry root biomass (DRB). To determine the plant height, a 5.0 m graduated scale was employed, measuring from the stem base to the main apical bud. The stem diameter was estimated at the plant using a digital caliper with 0.005 mm precision. To assess the fresh shoot biomass of the aerial plant parts (stem and leaves) and fresh root biomass, the plants were removed from the pots, and the roots disconnected from the top parts of the plants. Both parts were weighed separately utilizing a digital scale with 0.0001 g accuracy. Once the aerial parts and the roots were weighed, they were stored in paper bags, identified and placed under forced air circulation at 65 °C for a 72 hour period. They were later removed and re-weighed to record the dry biomass (stem and leaves) and dry root biomass. The number of leaves was directly counted.

Statistical analysis

The results were subjected to analysis of variance by F-test ($p < 0.05$); when significant, the averages were compared by Tukey's test at 1 % and 5 % of probability of probability, using the statistical software SISVAR (Ferreira, 2008).

Conclusion

Mineral fertilization provided the maximization of the growth parameters of cowpea plants in the vegetative phase. Mineral fertilization also promoted the maximum accumulation of phytomass in cowpea bean. The *manipueira* exerted a negative influence on the initial development of cowpea. However, more studies are required which will include adequate doses of this agro-industrial residue for use as an alternative crop fertilizer.

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