ISSN 1807-1929
Revista Brasileira de Engenharia Agrícola e Ambiental
Brazilian Journal of Agricultural and Environmental Engineering
v.28, n.6, e278380, 2024
Campina Grande, PB – http://www.agriambi.com.br – http://www.scielo.br/rbeaa

DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v28n6e278380

ORIGINAL ARTICLE

Curly lettuce production using cassava wastewater as a potassium source[1]

Produção de alface crespa utilizando água residuária da mandioca como fonte de potássio

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HIGHLIGHTS:

Lettuce's agronomic attributes decreased when plants were fertilized only with cassava wastewater as a source of potassium.
Lettuce's bromatological attributes were higher when plants were fertilized exclusively with cassava wastewater.
Cassava wastewater can be used as a complementary potassium source for lettuce, as it does not replace mineral fertilization.

ABSTRACT: Cassava wastewater (CWW) is a natural biofertilizer successfully tested as a source of K in long-cycle crops; however, it still needs to be better evaluated in short-cycle crops. This study aimed to evaluate the use of CWW as a source of K in the growth, yield, and physicochemical quality of curly lettuce. The study was conducted in a greenhouse, in a randomized block design arranged in a 5 × 2 + 1 factorial scheme, with four replicates. Five CWW doses were evaluated as an exclusive source of K: 0, 60, 120, 180, and 240 kg ha^{-1} of K_2O; two forms of application: 1-total dose at planting: 2-application of 50% of the dose at planting + 50% of the dose in topdressing, and an additional treatment: 100% mineral fertilization at 120 kg ha^{-1} of K_2O dose, using KCl as the source. The number of leaves (NL), plant height (PH), fresh mass (FM), dry mass (DM), moisture (MO), ashes (ASH), fibers (FB), lipids (LIP), proteins (PTN), and carbohydrates (CHO) contents were evaluated. All agronomic attributes of lettuce showed lower values when fertilized only with CWW than mineral fertilization. Lettuce fertilized only with CWW, depending on total or split application and dose, showed higher ASH, FB, LIP, PTN, and CHO contents reaching 4.05, 3.97, 1.75, 7.11, and 3.63%, respectively, compared to mineral fertilization. The CWW can potentially be used as a complementary K source in lettuce cultivation, as its exclusive use does not replace traditional mineral fertilization.

Key words: *Lactuca sativa* L., *Manihot esculenta* Crantz, biofertilizer, sustainability

RESUMO: Água residuária de mandioca (ARM) é um biofertilizante natural que foi testado com sucesso como fonte de K para culturas de ciclo longo; entretanto, ainda precisa ser melhor avaliado em culturas de ciclo curto. Este estudo teve como objetivo avaliar a utilização do ARM como fonte de K no crescimento, produção e qualidade físico-química da alface crespa. O estudo foi realizado em casa de vegetação, em delineamento de blocos casualizados, em esquema fatorial 5 × 2 + 1, com quatro replicações. Foram avaliadas cinco doses de ARM como fonte exclusiva de K: 0, 60, 120, 180 e 240 kg ha^{-1} de K_2O; duas formas de aplicação: 1-dose total no plantio: 2-aplicação de 50% da dose no plantio + 50% em cobertura, e um tratamento adicional: adubação 100% mineral com dose de 120 kg ha^{-1} de K_2O, utilizando KCl como fonte. O número de folhas (NF), altura da planta (AP), massa fresca (MF), massa seca (MS), umidade (UM), conteúdos de cinzas (CZ), fibras (FB), lipídios (LIP), proteínas (PTN) e carboidratos (CHO) foram avaliados. Todos os atributos agronômicos da alface avaliados apresentaram valores inferiores quando adubada apenas com ARM comparado à adubação mineral. A alface adubada apenas com ARM, dependendo da aplicação total ou parcelamento e da dose, apresentou maiores teores de CZ, FB, LIP, PTN e CHO atingindo 4,05, 3,97, 1,75, 7,11 e 3,63%, respectivamente, em comparação à adubação mineral. A água residuária de mandioca pode potencialmente ser utilizado como fonte complementar de K na produção de alface, pois seu uso único não substitui a adubação mineral.

Palavras-chave: *Lactuca sativa* L., *Manihot esculenta* Crantz, biofertilizante, sustentabilidade

• Ref. 278380 – Received 10 Sept, 2023
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• Accepted 18 Feb, 2024 • Published 26 Mar, 2024
Editors: Toshik Iarley da Silva & Walter Esfrain Pereira

Introduction

Lettuce (*Lactuca sativa* L.) is Brazil's most consumed leafy vegetable and one of the three most produced in the world. It can be grown all year round, in any region of the country, in small areas, and at low cost (Queiroz et al., 2017). In addition, it is a vegetable of great social importance, as it presents essential nutritional aspects for human consumption (Shatilov et al., 2019), as it is rich in fiber, vitamins, and mineral salts, in addition to having healing properties determined by its high content of antioxidants, mainly vitamin C and polyphenols (Materska et al., 2019).

It is a short-cycle vegetable that requires high amounts of mineral fertilizers to meet its needs, mainly N and K, which are obtained using highly soluble fertilizers complemented with manure from cattle or other organic compounds (Cecílio Filho et al., 2018; Souza et al., 2019). An organic byproduct generated during the manufacturing process of cassava (*Manihot esculenta* Crantz) flour that can be used as fertilizer is cassava wastewater (CWW), which, as it is rich in nutrients, improves the chemical quality of the soil (Fernandes et al., 2019; Bailón-Salas et al., 2021; Costa et al., 2021a).

High volumes of CWW are generated by the cassava flour industries in Brazil and around the world; however, this byproduct is generally discarded in unproductive areas of the soil or waterways (Souza et al., 2019; Malik et al., 2020; Oghenejoboh et al., 2021). Conversely, some studies have already been conducted showing the beneficial effects of CWW application on corn (Barreto et al., 2014; Ramos et al., 2018), *Capsicum chinense* (Costa et al., 2020), and lettuce (Costa et al., 2021b).

On lettuce, Santos et al. (2010) reported gains with CWW in lettuce leaf area, number of leaves, and fresh and dry leaf mass; however, the authors pointed out that despite CWW presents desirable properties as a source of nutrients if it is not used correctly, it can cause damage to both the soil and plants. Duarte et al. (2012) observed increased leaf area, plant height, and fresh and dry mass with CWW as a fertilizer for a lettuce crop if an adequate dose is used. Costa et al. (2021b) also studied the microbial activity of lettuce-cropping soil and plant responses. They observed increased plant variables, soil nutritional status, and soil microbial activities depending on CWW dose.

Despite the studies already done with lettuce cultivation, the knowledge about using cassava wastewater for crop fertilization still needs consolidation through studies focusing on the contents of the primary nutrients available when applying CWW. In this context, the hypothesis assessed in this study is that CWW can be used as a source of K in cultivating short-cycle vegetables; however, it can alter the quality of the plants produced. This study aimed to evaluate the use of CWW as a source of potassium in the growth, yield, and physical-chemical quality of curly lettuce.

Material and Methods

The study was carried out in a greenhouse in an experimental area located in the municipality of Uberaba, Minas Gerais (19° 39' 19" S and 47° 57' 27" W, at 795 m altitude) between October and December 2018.

The prevailing climate of the experimental region is classified as Aw-type (tropical savanna, wet), according to the updated Köppen's classification (Beck et al., 2018), presenting hot and rainy summers and cold and dry winters. The experimental region's annual rainfall, temperature, and relative air humidity averages are about 1,600 mm, 22.6 °C, and 68%, respectively (INMET, 2019).

The soil was of medium texture (220, 720, and 60 g kg^{-1} of clay, sand, and silt, respectively) and classified as Oxisol (Soil Survey Staff, 2014) with moderate A-horizon (Soil Survey Staff, 2014). The soil nutritional composition (0-0.2 m superficial soil layer) presented: pH (H_2O) 5.1; 0.4 mg dm^{-3} of P (Resin); 110 mmol$_c$ dm^{-3} of K; 60 mmol$_c$ dm^{-3} of Ca; 20 mmol$_c$ dm^{-3} of Mg; 50 mmol$_c$ dm^{-3} of aluminum (Al); the sum of bases (SB) of 80 mmol$_c$ dm^{-3}; 26.5% of bases saturation.

The soil had its pH corrected with dolomitic limestone (36.4% calcium oxide, 44% magnesium oxide, 99.9% neutralization power, and the relative power of total neutralization of 90.28%) to raise the base saturation to 70%. The soil mixed with the dolomitic limestone was incubated for 30 days and irrigated daily. After this period, CWW was added to the soil in the pots at the studied doses.

The randomized block design was used with four replications arranged in a 5 × 2 + 1 factorial scheme. Five doses of K (0, 60, 120, 180, and 240 kg ha^{-1} of K_2O) in volumes of CWW (1 kg of K_2O = 103.7 L of CWW) and two ways of CWW application (1 - all the doses at planting: 2 - 50% of the dose at planting plus 50% at topdressing 30 days after seedling transplanting) were evaluated. One additional treatment containing mineral K fertilization was employed with 200 kg ha^{-1} of KCl (120 kg ha^{-1} of K_2O). The experimental unit was composed of six pots containing one plant per pot.

The recommended dose of mineral fertilization for lettuce cultivation was determined based on soil analysis and the recommendations indicated by Ribeiro et al. (1999). About 300 kg ha^{-1} of P_2O_5 and 40 kg ha^{-1} of K_2O were applied at planting, and about 160 kg ha^{-1} of N and 80 kg ha^{-1} of K_2O, divided twice (50% + 50%), were applied at 15 and 30 days after planting. Mineral K fertilization (KCl) was applied only to the additional treatment. The CWW chemical composition was determined by the Labfert Ltd. soil analysis laboratory (Uberaba, Brazil) and presented in Table 1.

Curly lettuce cultivar Veronica (Sakata®) seeds were sown by placing one seed per cell in 128-cell expanded polystyrene trays previously filled with Bioplant® substrate. The experimental plots were installed in an arch model greenhouse in pots with a capacity of 13 dm³, 0.35 m wide at the top, 0.23 m wide at the

Table 1. Chemical composition of the cassava wastewater used in the present study

Element	Unit	Concentration	Element	Unit	Concentration
N	g L^{-1}	13.000	Zn	mg L^{-1}	5.861
K	g L^{-1}	8.000	Cu	mg L^{-1}	1.521
P	g L^{-1}	3.000	Mn	mg L^{-1}	0.232
Ca	g L^{-1}	0.012	Pb	mg L^{-1}	0.300
Mg	g L^{-1}	0.022	Ba	mg L^{-1}	1.300
Fe	mg L^{-1}	10.833	Ni	mg L^{-1}	0.198

bottom, and 0.28 m high, filled with 11 dm^3 of soil collected from the arable layer (0 – 0.2 m deep), then placed on a wooden support 0.15 m above soil level.

Eight days after sowing, when they had four to six completely expanded definitive leaves and 0.07 to 0.1 m in height, the lettuce seedlings were transplanted, placing one seedling in the center of each pot. Then, these pots were arranged in a row inside the greenhouse, with a spacing of 1 m between the rows and 0.5 m between each pot. After sowing the lettuce seedlings, the plants were irrigated between three and four times daily using a drip system.

The drip irrigation dose was calculated based on the reference evapotranspiration (ETo) estimated with data obtained by the IFTM weather station. The temperature values were collected daily, and reference evapotranspiration was calculated according to Hargreaves & Samani (1985). An irrigation depth of 7.5 mm day^{-1} (462 mL pot^{-1}) was estimated for an average temperature of 24.5 °C.

The number of leaves (NL) and the plant height (PH) were evaluated in the harvest conducted 45 days after the transplanting in all the plants that presented commercial patterns, in those without evidence of flowering and with maximum vegetative growth, which occurred in about 90% of transplanted seedlings.

All harvested plants were cut just below the basal leaves, close to ground level, and half of these plants were sent directly to the laboratory for fresh mass (FM) determination. Then, these plants were placed in an oven with forced air circulation at 65 °C for 72 hours or until they reached constant mass to determine the dry mass (DM).

After harvesting, the other half of the lettuce plants were taken to the IFTM Food Analysis Laboratory for moisture (MO) assessments through the percentage of plant water content calculated after sample drying. The ashes (ASH) were determined by calcining the sample in muffle at 550 ºC until light ash was obtained, and the fiber (FB) was obtained according to the gravimetric method after digestion in an acid medium using the methodology by the IAL (2008).

The lipids (LIP) were evaluated by the Soxhlet method (gravimetric) and based on the amount of material solubilized by the solvent. The carbohydrates (CHO) were evaluated by the Tillmans method. The protein (PTN) was determined through the Kjeldahl method by quantifying the nitrogen in the sample following the methodology described by the AOAC (2005).

The analyses of protein, crude fiber, and lipids were performed with dry samples, later calculating the indexes for fresh matter. The other analyses were conducted with fresh matter, 24 hours after harvesting, keeping it refrigerated during this period and packed in refrigerated five-layer nylon bags.

Extreme values (outliers) in all lettuce variables evaluated were identified using boxplot graphs of residuals generated using IBM SPSS® Statistics v. 20 (IBM Corp., Armonk, NY, USA). The outlier values identified were excluded from statistical analysis.

The data were submitted to analysis of variance ($p \leq 0.05$) using the Agroestat® software v. 1.1.0.626 (Barbosa & Maldonado Jr., 2015) after checking the assumptions with the Shapiro-Wilk test for normality and Levene's test for homogeneity of variance using the IBM SPSS Statistics software (IBM Corp., Armonk, NY, USA, v. 20), both with $p \leq 0.05$.

Then, the ways of CWW application were compared by the Tukey test. Regression analyses were applied for the CWW doses. Sigma Plot® v.12 (Systat Software Inc., San Jose, CA) was used to illustrate graphs.

Results and Discussion

Fresh mass (FM), dry mass (DM), moisture (MO), ashes (ASH), fiber (FB), lipids (LIP), proteins (PTN), and carbohydrates (CHO) were influenced by the interaction ($p \leq 0.05$) between the doses and application ways of cassava wastewater (CWW)(Table 2). Plant height and shoot dry mass were influenced ($p \leq 0.05$) only by K doses.

The initial lettuce growth evaluation at the time of harvest indicated that the number of leaves from the CWW doses was lower ($p \leq 0.05$) compared to the mineral fertilization with KCl (Figure 1); however, there were no differences between application ways (Table 2), nor significant model ($p > 0.05$) could be fitted to the data.

The results of the number of lettuce leaves indicate that the use of CWW is not promising when used as the only source of K, as this parameter is essential in the commercialization of the vegetable. This reduction in the number of leaves where CWW was applied could perhaps be explained by the high mobility of K in the soil and in the plant, as highlighted by Cecílio Filho et al. (2018) in their study, which may have caused a certain imbalance of nutrients in the pot (Grangeiro et al. 2006), as there were losses by leaching in the controlled environment. The same did not occur where K was applied via granulated mineral fertilizer.

Table 2. Summary of the assumptions of analysis of variance (probability) and analysis of variance (mean square) of the variables evaluated on lettuce plants

Assumptions		NL	PH	FM	DM	MO	ASH	FB	LIP	PTN	CHO
Normality		0.342	0.358	0.052	0.060	0.591	0.210	0.551	0.175	0.196	0.566
Homogeneity		0.279	0.624	0.550	0.921	0.103	0.044	0.141	0.003	0.061	0.160
Source of variation	**DF**										
D	4	0.0001^{ns}	1.0908**	125.10**	7.3799**	40.03**	2.5736**	0.2881**	0.0604**	1.4805**	7.6409**
P	1	0.2365^{ns}	0.0219^{ns}	22.9265^{ns}	4.6574^{ns}	15.31**	0.1178**	0.7322**	0.0115**	2.4607**	14.89**
D*P	4	0.0221^{ns}	0.2806^{ns}	324.80**	1.2144^{ns}	11.86**	0.9679**	0.9330**	0.0118**	0.8045**	11.94**
Ad*(D*P)	1	175.24**	275.84**	7,851.88**	152.18**	27.52**	0.6503**	0.1914**	0.0141**	0.0170^{ns}	4.7194**
Block	3										
Error	30										

ANOVA assumptions ($p < 0.01$) – Normality- Shapiro-Wilk's test for the normality of residues distribution; Homogeneity- Levene's test for homogeneous data variances. Lettuce variables – NL- Number of lettuce leaves; PH- Lettuce plant height; FM- Fresh mass; DM- Dry mass; MO- Plant tissue moisture; ASH- Plant tissue ashes; FB- Lettuce fiber; LIP- Lettuce lipid; PTN- Lettuce protein; CHO- Lettuce carbohydrates. ANOVA ($p \leq 0.05$) – DF - Degrees of freedom; D- Cassava wastewater dose; P- Application ways; Ad- Additional treatment (mineral K fertilization). ** - p-value significant at 0.01 ($p \leq 0.01$). ns - Non-significant ($p > 0.05$) by F test

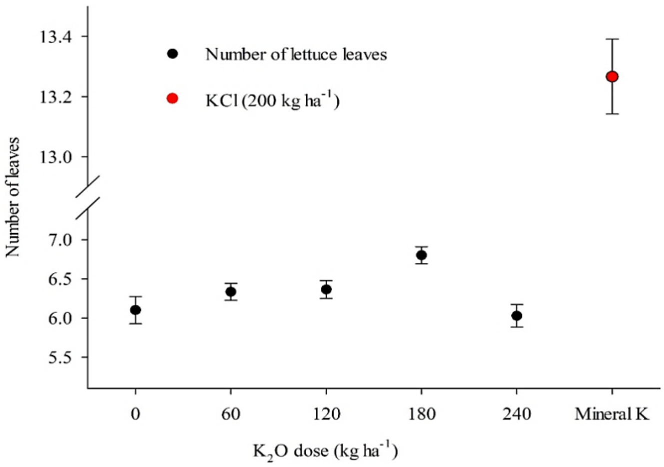


Figure 1. Number of leaves of lettuce plants at harvest time according to the K_2O doses using cassava wastewater (CWW) as the source

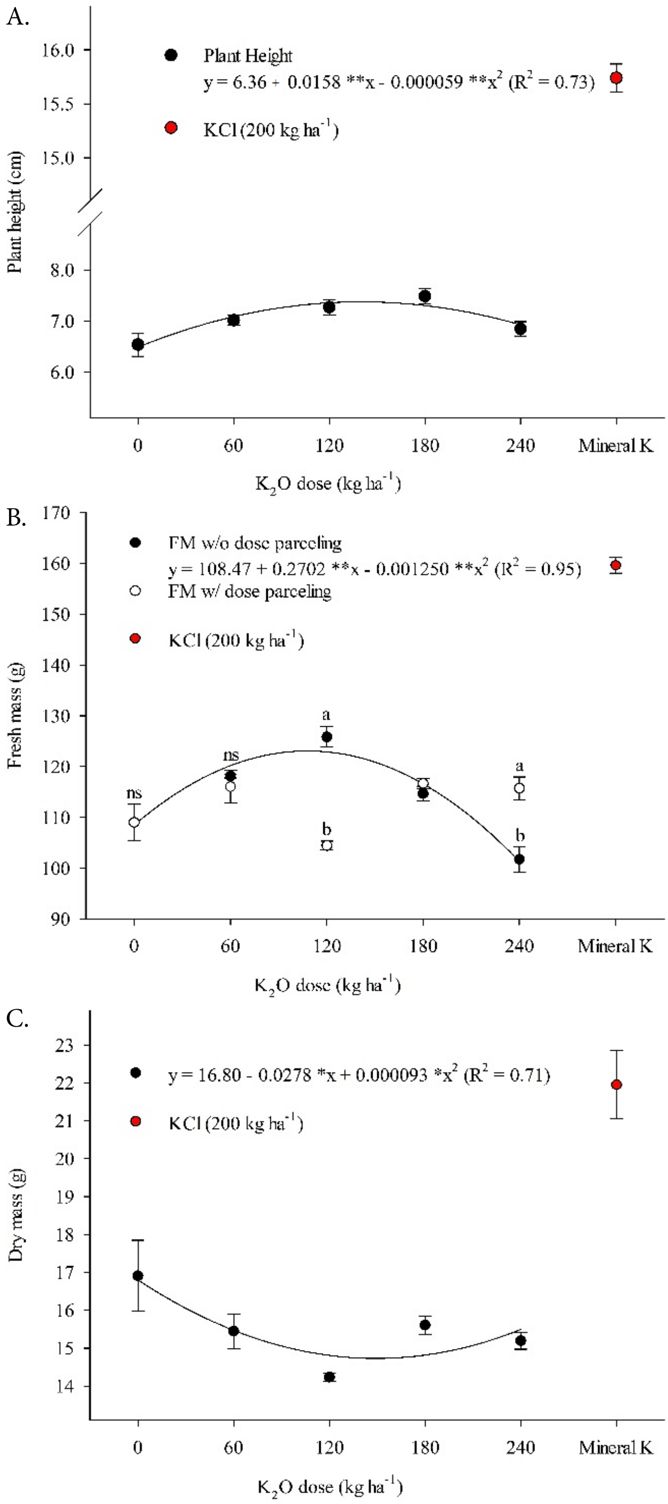
According to Taiz & Zeiger (2017), it is expected to occur a reduction in the NL and the FM of the vegetable when a product with a predominance of K ion among its constituent elements and in high doses is applied. Such a product can cause an imbalance of basic cations in the soil due to saturation by this element and predisposition to calcium (Ca) and magnesium (Mg) leaching. When applied in low doses, it can cause K deficiency, which directly interferes with the photosynthetic activity of plants, as it is an element recognized as a pronounced enzymatic activator, according to Marschner (2012).

In a similar study with lettuce, Santos et al. (2010) applied different doses of CWW and observed that increasing doses provided an increasing number of lettuce leaves until the maximum response point of 395.85 mL, which provided an estimated value of 35.67 leaves; however, in doses above this maximum point, there is a decrease in the number of lettuce leaves, a value similar to that found in this study for the highest dose.

The interaction of factors (application ways and K_2O doses) was interpreted with a focus on the best-fitted regressions for the respective lettuce variables. The justification for not exploring the statistical interaction lies in the observed inconsistency in performance between the two application ways at different K_2O doses. In one K_2O dose, a particular application demonstrated superior results, while in the subsequent K_2O dose, a distinct application exhibited the optimal outcome. Therefore, evaluating each application way for the significant interactions at different K_2O doses may provide a more accurate understanding of their contributions to overall lettuce performance.

The regression analysis of CWW doses for plant height showed a quadratic fit, with the increase in CWW doses providing a significant increase in plant height up to the estimated dose of 134 kg ha^{-1} of K_2O, which reached the maximum value of 7.41 cm (Figure 2A), from which the values decreased. It should be added that there were no significant differences ($p \leq 0.05$) between the application ways.

For FM of lettuce, the regression curve showed a quadratic fit when the dose was all applied at planting, showing that increasing the dose of CWW caused a positive influence on FM production until reaching the estimated dose of 123 kg ha^{-1} of K_2O and 108 g per plant, from which there was a slow decrease as doses increased. For the application of the split dose, the curve fit was also quadratic up to the estimated dose of 45 kg ha^{-1} of K_2O. Growth was slow, and the plant reached an FM production of 110.36 g per plant. However, from this point on, the increase in CWW doses also increased the availability of



* - p-value significant at 0.05 ($p \leq 0.05$). ** - p-value significant at 0.01 ($p \leq 0.01$). ns - Non-significant ($p > 0.05$). Letters in the same K_2O dose indicate differences ($p \leq 0.05$) between application ways

Figure 2. Height (A), fresh mass (B), and dry mass(C) of lettuce plants at harvest time according to the K_2O doses using cassava wastewater (CWW) as the source

nutrients in the plant, which increased FM up to the highest applied dose (240 kg ha^{-1} of K_2O), reaching a maximum value of 118.6 g per plant (Figure 2B).

The FM had a maximum value of 160 g per plant in the treatment with K applied via mineral fertilizer, significantly higher than those observed in the K doses used via CWW (Table 2, Figure 2B).

Concerning DM, the regression curve showed a quadratic fit, as there was a decrease in DM as the K doses were applied via CWW until reaching the minimum value of 14.72 g per plant at the dose estimate of 149 kg ha^{-1} of K_2O from which the DM value increased to reach 16.89 g per plant at the highest dose (240 kg ha^{-1} of K_2O). In the treatment with K applied via mineral fertilizer, the minimum DM was 19.91 g per plant, significantly higher than those observed in the K doses applied via CWW (Table 2, Figure 2C).

Plant height (Figure 2A) and fresh (Figure 2B) and dry (Figure 2C) mass were not influenced by the application ways. In addition, the results observed for these variables were well below the results observed for lettuce fertilized with mineral K. The tallest plant (7.42 cm) and the one with the least mass (14.22 g) were observed, respectively, with the application of 133.90 and 149.46 kg ha^{-1} of K_2O via CWW. These results are 52.86 and 39.10% lower than the observed results (15.74 cm and 23.35 g) when mineral K was used, respectively.

The lettuce FM (Figure 2B) and DM (Figure 2C) in treatments where total CWW dose was applied at planting (116.75 and 14.97 g per plant) and split (117.17 and 15.47 g per plant) were significantly lower than those obtained where mineral fertilization was applied (177.27 and 23.35 g per plant), respectively. In the same way, the average NF of lettuce (28 leaves) in the treatments that received the doses of CWW were inferior compared to the values obtained in the mineral fertilization (35 leaves).

The quadratic adjustment of the regression curve for the FM of lettuce when the dose was applied at planting was highly significant (R^2 = 0.97); thus, it can be inferred that the model used safely explains the maximum point of FM accumulation of 108 g per plant at the estimated dose of 123 kg ha^{-1} of K_2O, which is close to the ideal dose of 120 kg ha^{-1} of K_2O recommended by Ribeiro et al. (1999) for lettuce cultivation, and the same did not occur when the CWW dose was split (R^2 = 0.61) (Figure 2B)

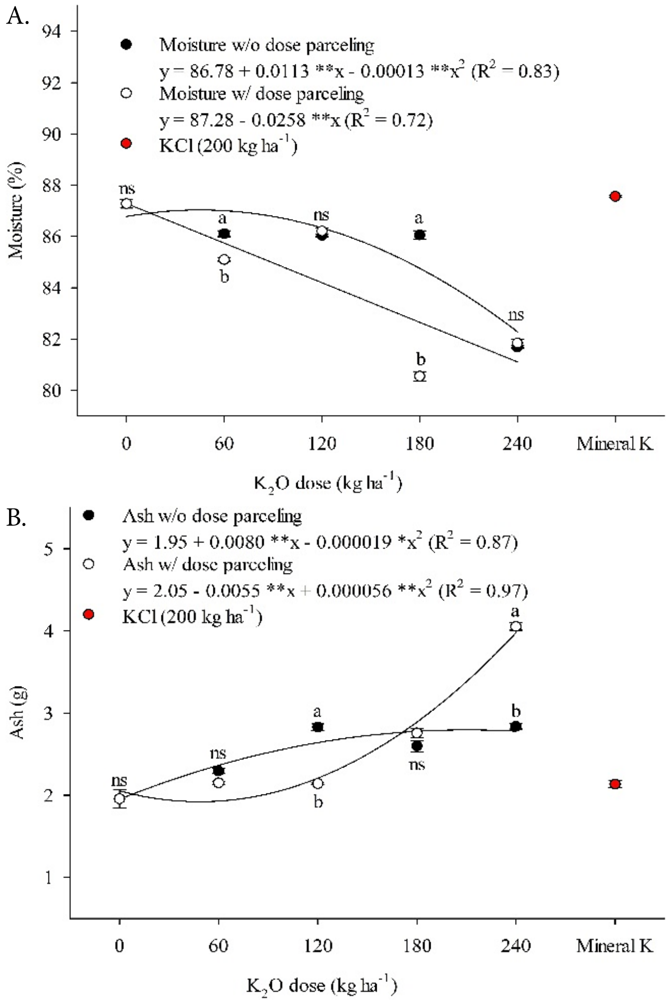
The results found in the research for the number of leaves, PH, FM, and DM indicate that CWW can be used as a positive complement for the production of the lettuce crop and not as a substitute for mineral fertilization. Duarte et al. (2012) reported positive biometric responses of lettuce to the application of CWW with doses equivalent to those of the present study since the authors evaluated doses from 12 to 155 kg ha^{-1} of K_2O, different from the results found in the present study for the number of leaves.

Santos et al. (2010) also found a positive effect of CWW application on plant height and observed the highest value of 16.67 cm with a dose of 138.20 kg ha^{-1} of K_2O. In addition, they verified that doses above 207.3 kg ha^{-1} K_2O reduced lettuce leaf area compared to control (without CWW application). This decrease in leaf height with increasing CWW doses also occurred in this study; however, not significant ($p \leq 0.05$), which may be explained by the difference between the doses being lower.

In their study, Grangeiro et al. (2006) confirmed the high P requirement of lettuce in quantitative terms, as it is a culture that absorbs high concentrations in a short period due to its shorter cultivation cycle of 45 days until harvest, a condition also observed in this study, because in the control treatment the lowest values were observed for most of the evaluated attributes.

The regression analysis for plant MO (water content) showed a decreasing linear fit when the entire dose of CWW was applied at planting (Figure 3A), which reached the minimum value of 81.25% moisture at the dose of 240 kg ha^{-1} of K_2O. In contrast, in the treatments in which the dose was split, the regression curve fit was quadratic because the leaf moisture increased until reaching 87.02% at the estimated dose of 43 kg ha^{-1} of K_2O, from which the values decreased until the plant reached the minimum of 82.15% moisture at the highest dose of CWW used. In the treatment with mineral fertilizer, the MO obtained was 87.82%.

The MO of lettuce observed in the present study varied between 81.25 and 87.82%, lower than the 94 to 95% reported by Stertz et al. (2005) and 95.81% by Pereira et al. (2016). In



*: p-value significant at 0.05 ($p \leq 0.05$). **: p-value significant at 0.01 ($p \leq 0.01$). ns: non-significant ($p > 0.05$). Letters in the same K_2O dose indicate differences ($p \leq 0.05$) between application ways

Figure 3. Leaf moisture (A) and ash (B) of lettuce plants at harvest time according to the K_2O doses using cassava wastewater (CWW) as the source

their study, Ohse et al. (2009) also identified leaf moisture ranging from 95.01 to 96.03% in evaluating five different lettuce varieties. On average, plant water content was about 8-10% lower than lettuce moisture reported in the literature.

As the control treatments (0 kg ha^{-1} K_2O as CWW) and mineral fertilization with KCl as a source of K showed similar plant moisture (87.82%), it can be assumed that the plants were not at the ideal stage for the evaluation of the variable, even so, the dose of CWW equivalent to 240 kg ha^{-1} of K_2O, with or without splitting the dose, significantly reduced ($p \leq 0.05$) the water content of the plant compared to the control or mineral fertilization.

Regarding the ash content, the regression analysis showed a quadratic adjustment curve for the two application ways (Figure 3B). When the entire dose of CWW was applied at planting, there was an increase in the ash content until reaching the maximum value of 2.79 g at the estimated dose of 210.52 kg ha^{-1} of K_2O, from which point it began to decline. In the split dose treatments, the opposite effect occurred, as the amount of ash decreased until reaching the minimum value of 1.94 g at the estimated dose of 49.11 kg ha^{-1}, from which it increased exponentially until reaching the maximum value of 4.18 g at the highest dose evaluated. In the treatment with mineral fertilizer, the ash content reached a maximum value of 2.15 g.

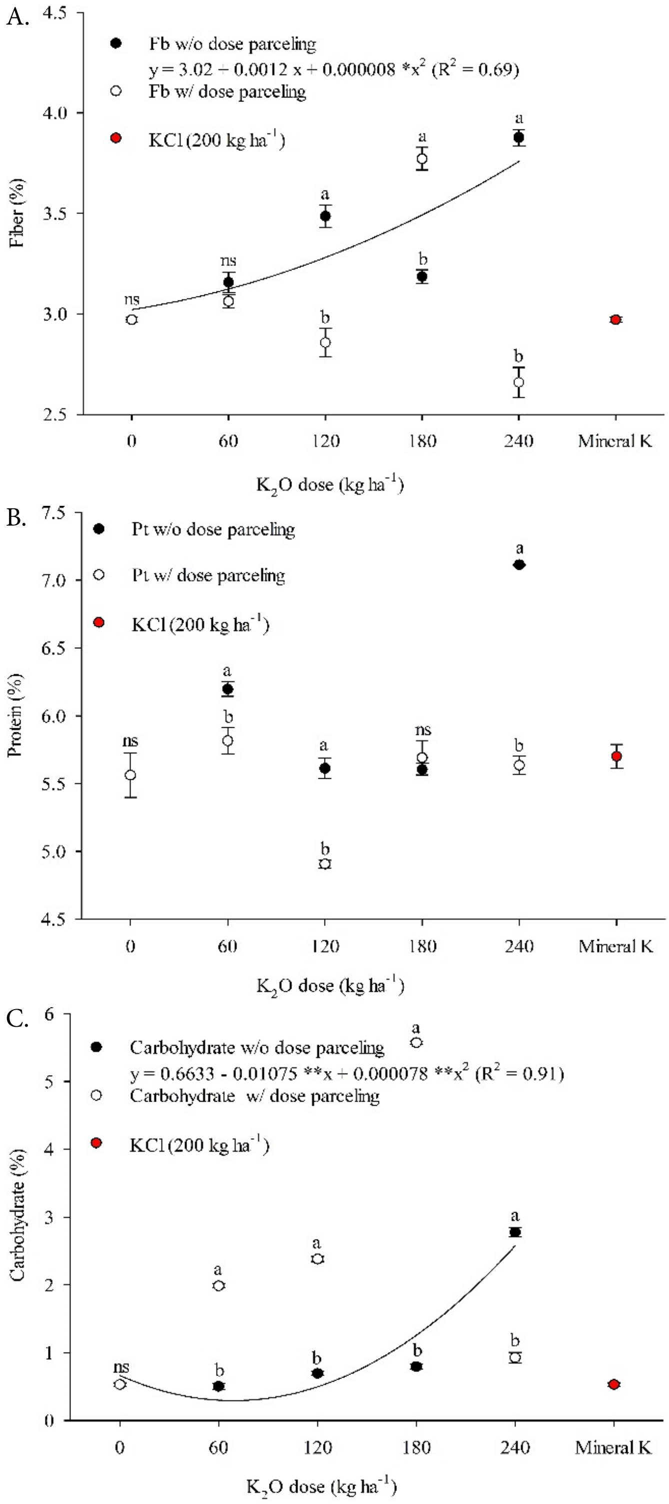
The ASH content (Figure 3B) showed similar results between the control (1.95%) and mineral K (2.15%); however, the ash content increased with the increase in the CWW doses used, reaching 4.05% when the dose was split into two applications.

The plant ash content reflects the quantity of minerals present in a sample. Cassava wastewater as a source of K favored the increase of ASH compared to mineral fertilization. As observed, doses of 180 and 240 kg ha^{-1} of K_2O as CWW (with or without splitting) presented significantly higher ($p \leq 0.05$) ash contents. This increase in ash content is related to the lower moisture of lettuce compared to the treatment with mineral fertilizer. It can be considered a nutritional improvement for the consumer.

The result observed for the ash content of lettuce (4.05%) when 240 kg ha^{-1} of K_2O was applied using CWW, divided into two applications, was three times greater than the result for ash observed by Pereira et al. (2016) (1.35%) in lettuce produced commercially and sampled at a street market. The ASH content of lettuce indicates that there was an effect of mineral concentration due to the lower growth of the plant with the application of CWW on lettuce production.

For the FB content, through the regression analysis, a maximum value of 3.97% was observed when the CWW dose was applied in the planting. In contrast, for the treatment with the split dose, the maximum FB content was 3.15% at the estimated dose of 75 kg ha^{-1} of K_2O, from which the values decreased until 2.66 g at the highest dose. In contrast, the maximum FB content in the treatment with mineral fertilizer was 2.90% (Figure 4A).

The FB contents in the present study, mainly when CWW was used as a biofertilizer, were higher than those reported by Ohse et al. (2009), who found levels ranging from 0.61 to



*: p-value significant at 0.05 ($p \leq 0.05$). **: p-value significant at 0.01 ($p \leq 0.01$). ns: non-significant ($p > 0.05$). Letters in the same K_2O dose indicate differences ($p \leq 0.05$) between application ways

Figure 4. Lettuce leaf content of fibers (A), protein (B), and carbohydrates (C) of lettuce plants at harvest time according to the K_2O doses using cassava wastewater (CWW) as the source

0.77% in different lettuce genotypes. According to the author, the FB content has high commercial importance since fibrous lettuce leaves are generally not desirable, as these leaves are only used in hot dishes, such as hamburgers, to maintain freshness and crispness.

When the entire dose of CWW was applied at planting, the LIP content increased linearly with increasing doses until reaching a maximum of 1.75% at the highest dose used. The same occurred for split doses, which reached a value of 1.77% at the highest dose; however, the curves were not adjusted in the regression analysis in both cases.

Lettuce is a central source of fiber for the human diet and a vegetable with low LIP content (low calories) (Somerville et al., 2000). Stertz et al. (2005) reported 0.29% LIP content in curly lettuce, while Ohse et al. (2009) observed variations from 0.18 to 0.42% LIP content in five distinct lettuce genotypes. Those are low lipid contents in lettuce compared to the results observed in the present study (1.56 - 1.77%), probably related to the genotype studied since both the control and mineral K fertilization presented high lettuce lipid content (1.62%).

Regarding the PTNs, the treatment with the application of the total dose, the regression curve showed a quadratic fit, with the PTN value decreasing to the minimum value of 5.59% at the estimated dose of 75 kg ha^{-1} of K_2O (Figure 4B). From this point, it increased to the maximum value of 7.11% at the dose of 240 kg ha^{-1} of K_2O applied as CWW. The same occurred in the treatments where the fertilization was split, as the PTN content decreased until reaching 5.29% at the estimated dose of 120 kg ha^{-1} of K_2O, and from this value, it increased until reaching the content of 5.63% at the dose of 240 kg ha^{-1} of K_2O; however, there was no curve fitting with a coefficient of determination (R^2) greater than 60%.

The lettuce protein content observed in the present study (cv. Veronica) varied from 5.26 to 6.37%, above that in other studies. This high protein content in lettuce is favorable for human lettuce consumption. Stertz et al. (2005) reported 1.10 to 1.33% of the protein in lettuces cultivated in conventional, organic, or hydroponic systems. Ohse et al. (2009) registered protein contents ranging from 0.83 to 1.28%. The protein content observed in the present study and those observed in the literature indicate a significant variability among lettuce cultivars and cropping systems. In addition, the protein content of mineral K fertilization (source of KCl) did not differ from the CWW treatments, demonstrating that lettuce protein content is a very stable plant trait, and crop management can have little impact on this variable.

For the CHO values, the regression curves also showed a quadratic adjustment (Figure 4C). When K was applied at the total dose at planting, the minimum value reached was 0.30% at the estimated dose of 69 kg ha^{-1} of K_2O. In contrast, for divided doses, the values increased to a maximum of 3.63% at the estimated dose of 137 kg ha^{-1} of K_2O, then gradually decreased until the content was 2.90%.

Applying any of the K doses studied using CWW (with or without splitting) increased the CHO content of lettuce. This result is promising, as the accumulation of sugars is related to a more protective condition (abiotic and biotic stresses) for the plant (Ciereszko, 2018). The increase in the CHO content observed in the plants in this study is probably related to the lower growth of the plant regarding mineral fertilization, thus providing a concentration effect in the tissues.

The soil used for this study is sandy (72%) and has a lower nutrient reserve (low clay content) for plant growth during the crop cycle. Furthermore, lettuce is highly nutrient-demanding during its short crop cycle; thus, CWW - a biofertilizer - can contribute to soil fertility. However, it is necessary to study the quantity to be applied so that the application of the biofertilizer does not cause the leaching of nutrients.

The nutritional content of cassava flour processing (wastewater) is significant but varies significantly among the sources (Costa et al., 2021b). As previously discussed, soils with low clay content may benefit most from CWW application; thus, soil conditions (physical and chemical) and nutritional nutrient concentration of the applied CWW will likely influence lettuce plant characteristics. In addition, the low soil K availability - or the application of K sub-doses - can (i) cause a direct effect on the photosynthesis metabolism, (ii) affect the osmoregulation of the guardian cell of the stomata, and (iii) play as an enzymatic activator (Sardans & Peñuelas, 2021); thus, affecting the plant responses to the fertilization.

The effects observed by applying CWW doses are not just a consequence of the K present in CWW. Other nutrients were also present in the CWW, which can contribute to the observed results. However, CWW is still a promising crop alternative that can improve plant responses and a sustainable treatment for environmental responsibility when used as a crop fertilizer. However, based on the results observed in this study and others in the literature, CWW should complement the lettuce plant nutrition protocol and not replace common concentrated mineral fertilizers. New studies including CWW as a component of organomineral fertilizers, for example, may generate great positive results for the lettuce crop.

The results obtained in this study confirm the hypothesis assessed, as there were significant differences ($p \leq 0.05$) between the doses of CWW applied throughout the planting and split twice. There were also substantial changes in the agronomic (MO, FM, and DM) and physical-chemicals (ASH, CF, LIP, PTN, and CHO) attributes compared to CWW and mineral fertilizer.

Conclusions

1. All the agronomic attributes of lettuce showed lower values when fertilized only with cassava wastewater (CWW) compared to mineral fertilization.

2. Lettuce fertilized only with CWW, depending on total or split application and dose, showed higher ash, fiber, lipid, protein, and carbohydrate contents (reaching 4.05, 3.97, 1.75, 7.11, and 3.63%, respectively) compared to mineral fertilization.

3. The CWW has the potential to be used as a complementary source of K in lettuce production, as its exclusive use does not replace traditional mineral fertilization.

Acknowledgments

To the Instituto Federal do Triângulo Mineiro, Campus Uberaba, for providing the necessary equipment and laboratory space to conduct the experiments and analyses. To the National Council for Scientific and Technological Development (Process 306151/2020-0), for granting Scientific Initiation Scholarships to the students involved with the project.

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