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GRAIN YIELD AND MICROBIOLOGICAL QUALITY OF COWPEA PLANTS GROWN UNDER RESIDUAL EFFECT OF SEWAGE SLUDGE FERTILIZATION[1]

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ABSTRACT - Cowpea plants produce protein-rich grains and present high yield potential when grown under irrigation and organic fertilization, enabling to substitute part of the mineral fertilizer with sewage sludge, reducing costs and generating environmental benefits. Thus, a field study about residual effect of sewage sludge fertilization on cowpea was developed to evaluate this substitution. The experiment was conducted using a randomized block design with four replications, in a 2×5 factorial arrangement consisted of two fertilization types (residual effect of sewage sludge fertilization, and mineral fertilizers) and five cowpea cultivars (BRS-Pajeu, BRS-Xiquexique, BRS-Marataoa, BRS-Pujante, and BRS-Cauame). The residual fertilizations were from applications of sewage sludge and NPK mineral fertilizers for pineapple crops grown in the area before the cowpea crop. The experiment was conducted using 80,000 plants ha^{-1} and micro-sprinkler irrigation. Vegetative and yield components, grain yield, and microbiological quality were analyzed. BRS-Pujante had the highest cowpea grain yield (4,124 kg ha^{-1}) and the highest means for vegetative and yield components. The soil with residual sewage sludge fertilization improved the cowpea root growth and grain yield (3,854 kg ha^{-1}); the latter was 19% higher than that of treatments with mineral fertilization. These results were related to the great soil organic matter content provided by the sludge fertilization, which promotes nutrient mineralization, increasing grain yield. Fresh and dry grains of cowpea plants grown under residual effect of sewage sludge fertilization have similar microbiological quality to those of plants grown under mineral fertilization.

Keywords: *Vigna unguiculata (L)*. Biosolid. Coliform. Plant nutrition.

PRODUÇÃO E QUALIDADE MICROBIOLÓGICA DE CULTIVARES DE FEIJÃO-CAUPI APÓS ADUBAÇÃO RESIDUAL COM LODO DE ESGOTO

RESUMO - O feijão-caupi é um cereal rico em proteínas que apresenta elevado potencial produtivo quando cultivado com irrigação e adubação orgânica, sendo possível substituir parte dos fertilizantes minerais pelo lodo de esgoto, com redução de custos e vantagens ambientais. Para avaliar essa substituição, desenvolveu-se um estudo da adubação residual com lodo de esgoto em feijoeiros cultivados no campo, no delineamento em blocos casualizados com quatro repetições, em esquema fatorial 2 x 5, correspondendo a dois tipos de adubação (efeito residual do lodo de esgoto ou fertilizantes minerais), e cinco cultivares de feijão-caupi (BRS Pajeú, BRS Xiquexique, BRS Marataoã, BRS Pujante e BRS Cauamé). A adubação residual foi caracterizada pela aplicação de lodo e fertilizantes minerais NPK no cultivo antecessor (abacaxizeiro) ao feijão-caupi. As parcelas foram cultivadas com 80.000 plantas ha^{-1} de feijão-caupi, sendo irrigadas com microaspersão. Foram caracterizados os componentes vegetativos, componentes de produção e a produtividade dos feijoeiros, além da qualidade microbiológica. A cultivar BRS Pujante apresentou maiores médias para os componentes vegetativos e reprodutivos implicando em maior produtividade de grãos, com 4.124 kg ha^{-1}. O solo adubado com lodo de esgoto propiciou maior crescimento radicular e produtividade de feijão-caupi (3.854 kg ha^{-1}), 19% superior àqueles que receberam somente adubação mineral residual. Esses resultados foram atribuídos ao maior teor de matéria orgânica no solo adubado com lodo, propiciando ambiente favorável para a mineralização de nutrientes e maior produção de grãos. Os grãos verdes e secos de feijão-caupi produzidos após a adubação com lodo de esgoto apresentam qualidade microbiológica semelhante àqueles obtidos no cultivo com adubação mineral.

Palavras-chave: *Vigna unguiculata (L)*. Biossólido. Coliformes. Nutrição de plantas.

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INTRODUCTION

Cowpea (*Vigna unguiculata*), also known as black eyed pea in the USA, is traditionally known as Gorutubano bean in the semiarid region of the state of Minas Gerais, Brazil; it presents high yield potential when grown under ideal conditions of soil fertilization and water availability, and when is from a good genetic material (BRITO et al., 2012; MONTEIRO et al., 2012). Cowpea is typically grown by family farmers; it is an excellent source of proteins, essential amino acids, carbohydrates, vitamins, and minerals, mainly for low-income populations (SILVA et al., 2018a; CONAB, 2018).

The Brazilian cowpea production in the 2017/2018 crop seasons was 863,200 Mg (equivalent to 25.4% of the common bean production), over an area of 1,533,100 hectares, presenting yield of 563 kg ha^{-1} (CONAB, 2018). The state of Minas Gerais had a production of 7,700 Mg, with mean yield of 551 kg ha^{-1} (CONAB, 2018). These results show its low mean yield in Brazil and in Minas Gerais when compared to its productive potential, which can reach more than 4,000 kg ha^{-1} (SILVA et al., 2018b). This is probably due to the use of low technological level (MONTEIRO et al., 2012), unbalanced fertilization, and non-adapted cultivars to the local conditions.

Several factors affect the cowpea plant growth and yield, such as environmental and genetic conditions (LOCATELLI et al., 2014) and irrigation (BLANCO et al., 2011; SANTOS, 2011) and mineral and organic fertilization managements (BRITO et al., 2012), considering that fertilization stimulates nitrogen fixation by *Rhizobium* sp. in symbiosis with cowpea plants (PAMPANA et al., 2017). Therefore, fertilization management strategies are necessary to maintain the productivity of agricultural areas, and a sustainable alternative is the use of sewage sludge as soil fertilizer and conditioner (ALBUQUERQUE et al., 2015).

Sewage sludge is a viable alternative nutrient source for plants (ALBUQUERQUE et al., 2015), providing residual effect for succeeding crops because of its slow nutrient release that can last several years (SIEBIELEC; SIEBIELEC; LIPSKI, 2018). This effect can allow a reduction in the consumption of mineral fertilizers and crop implementation costs.

Sewage sludge can be used as soil fertilizer for crops, such as common bean (KUMAR; CHOPRA, 2014), rice (LATARE et al., 2014), sunflower (FREITAS et al., 2012), sugarcane (FRANCO et al., 2010), and wheat (LOBO; GRASSI FILHO; KUMMER, 2014). The application of 100 Mg ha^{-1} of sewage sludge resulted in higher grain yield of maize (521%), wheat (129%), barley (124%), and rape (150%) when compared to crops without this fertilization (SIEBIELEC; SIEBIELEC; LIPSKI, 2018). The residual fertilization from application of 40 Mg $ha^{-1}$of sewage sludge to the soil for rice crops resulted in 68% higher grain yield for the subsequent wheat crops when compared to the treatment with no sewage sludge fertilization, and also in higher yield than the wheat under mineral fertilization (NPK) (LATARE et al., 2014). However, the presence of microbiological contaminants can hinder the application of sewage sludge to the soil for grain and fruit crops that are fresh consumed by humans, mainly when considering the presence of coliforms and thermotolerant microorganisms. The higher contamination risk to plants is soon after the sewage sludge application to the soil, and its exposition to solar radiation during this period can eliminate the contamination risk to grains and fruits.

Microbiological contaminations at harvest and post-harvest can occur due to different factors, especially the inadequate handling of grains, presence of domestic animals, and contamination of transporting equipment, cleaning waters, and packaging. Therefore, greater care is required in agricultural systems that use sewage sludge, mainly at harvesting, application of post-harvest products, and choosing of irrigation system (ALVES et al., 2017).

The main pathogenic agents in sewage sludges that can contaminate cowpea plants are bacteria, protozoa, fungi, virus, and helminths, whose presence and quantity depend on the origin, season of the year, and treatment process used for the sewage sludge (MOTA et al., 2018). Regarding human health, the contamination risk is related to the handling or consumption of inadequately clean fresh foods. Although the cowpea is usually cooked at high temperatures and seldom presents direct microbiological contamination through ingestion.

In this context, the objective of this work was to evaluate the residual effects of fertiization with sewage sludge and mineral fertilization on vegetative characteristics, yield components, and microbiological contamination of irrigated cowpea plants grown under the climatic conditions of the semiarid region of the state of Minas Gerais, Brazil.

MATERIAL AND METHODS

The study was conducted in the summer-autumn period, known as dry crop season, in the municipality of Janaúba, MG, Brazil (15º43'47.4", 43º19'22.1", and altitude of 540 m). The climate of the region is Aw, tropical with dry winter, according to the Köppen classification, with average annual rainfall depth of 850 mm concentrated in November to March. The soil of the experiment area was classified as Oxisol (Latossolo Vermelho Eutrófico; EMBRAPA, 2014). The temperatures during the experiment period are described in Figure 1.

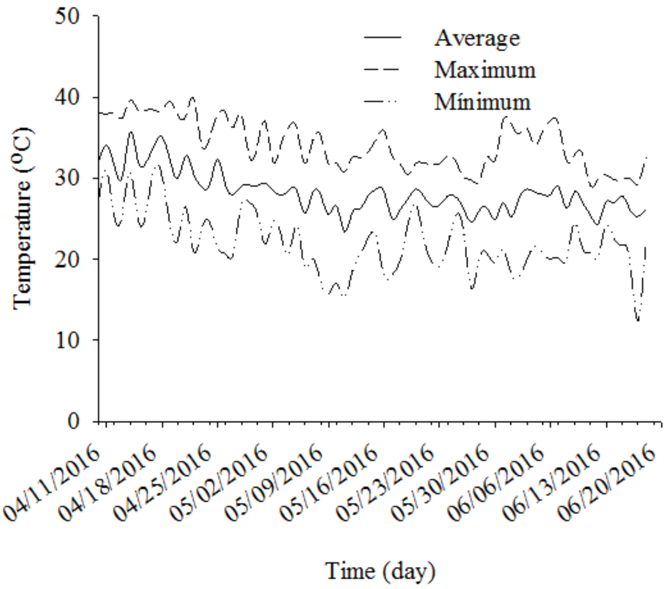

Figure 1. Average, maximum, and minimum air temperatures in the experimental area during the cowpea crop cycle, from April 10 to June 18, 2016.

The experimental area had been used for pastures (low quality and degraded) from 2004 to 2014; then, an experimental pineapple crop was conducted from 2014 to 2016, with application of sewage sludge and mineral fertilizers. P_2O_5 (simple superphosphate) were applied at pineapple planting in 2014, at a rate of 3 g per plant (SOUZA et al., 1999) to all plots. Sewage sludge as applied to the plots at a rate of 2.160 g per plant, considering a stand of 76,923 plants ha^{-1}. The N demand for the crop development (15 g per plant) was applied according to the results obtained by Cardoso et al. (2013).

The sewage sludge used (Table 1) was from the sewage treatment station of Janaúba, MG, Brazil. The sewage was first treated through a grid and a sand remover, then through a Parshall flume with ultrasonic flow meter, up flow anaerobic sludge blanket (UASB), one facultative pond, and two maturation ponds in series, with treatment capacity with continuous flow of up to 48.4 L s^{-1}.

The sewage sludge rate was calculated according to recommendations of the Brazilian National Council for Environment (Resolution 375; CONAMA, 2006), based on its nitrogen contents in mineral form ($N\text{-}NH_4^+$ + $N\text{-}NO_3^-$), and on a mineralizable organic nitrogen fraction of 20% for anaerobically digested sewage sludge, with application on the soil surface. The plot with mineral fertilizers (NPK) were applied at rate of 15 $gplant^{-1}$of N and K_2O, divided into four applications with 60-day intervals (MOTA et al., 2018).

Table 1. Chemical properties of the sewage sludge used in the experimental area.

pH H_2O	[1]OC	[2]N	[3]P	[3]K	[3]Ca	[3]Mg	[3]S	[3]Na	
					g kg^{-1}				
5.4	180.0	33.0	7.6	3.2	13.0	2.3	11.8	0.1	
[3]Zn	[3]Fe	[3]Mn	[3]Cu	[3]B	[3]Cd	[3]Pb	[3]Cr	[3]Ni	C/N
				mg kg^{-1}					
950.0	24293.0	152.0	14.0	5.8	5.2	69.1	143.2	22.1	5.5

[1]Organic carbon, Walkley-Black method; [2]Kjeldahl method; [3]Total contents extracted by nitro-perchloric solution.

After the pineapple harvest in 2016, the plants were removed from the experimental area, and the cowpea crop was implemented, using the same plots previously treated with sewage sludge and mineral fertilization.

The cowpea experiment was conducted using a randomized block experimental design with four replications, in a 2×5 factorial arrangement consisted of two fertilization types (residual effect of sewage sludge fertilization SS; and mineral fertilizers MF), and five cowpea cultivars (BRS-Pajeu, BRS-Xiquexique, BRS-Marataoa, BRS-Pujante, and BRS-Cauame). The plots had 7 three-meter plant rows and a total area of 6 m^2; the three central rows with ten plants per row were considered for evaluation. The characteristics of the cultivars used in the study are described in Table 2.

Table 2. Agronomical characteristics of the cowpea cultivars used in the study.

Cultivar	Commercial class	Growth habit	100-grain weight (g)	Pod length (cm)	Mean grain yield (kg ha^{-1})*	Recommended region(Brazil)
BRS-Pujante	Sempre-verde	Semi-prostate	24.8	18.4	1057.0	São Francisco Valley
BRS-Pajeu	Mulato	Semi-prostate	21.0	21.4	1035.0	North, Northeast, and Central-West
BRS-Xiquexique	Branco	Semi-prostate	16.5	20.0	1254.0	North, Northeast, and Central-West[1]
BRS-Marataoa	Sempre-verde	Semi-prostate	15.5	18.0	831.0	States of Piauí, Paraíba, and Bahia
BRS-Cauame	Branco	Semi-ereto	17.0	17.2	1060.0	North, Northeast, and Central-West[1]

*General mean of assays for the register of the cultivar; [1]Cultivar recommended for specific states.

The soil preparation consisted of manual cleaning and furrowing. The cowpea seeds were sowed on 03/10/2016 using a manual seeder, considering a depth of 3 to 4 cm, spacing of 0.5 m between rows and 0.25 m between plants, and a density of 8 plants m^{-2}.

A mineral fertilization for the cowpea crop was done after a soil chemical analysis (Table 3)at the end of the pineapple crop, according to the recommendations of Andrade Júnior et al. (2002). In the treatments MF and SS,60 and 20 kg ha^{-1} of P_2O_5 (simple superphosphate), respectively, were applied in the planting furrows; 21 kg ha^{-1} of K_2O (potassium sulfate) was applied in all treatments, divided into two topdressings at 15 and 25 days after emergence (DAE); and 111 g ha^{-1} of Mo (ammonium molybdate) was applied through foliar fertilization between 15 and 25 DAE. The plants were thinned at 10 DAE, keeping 80,000 plants ha^{-1}.

Table 3. Chemical attributes in the layers of 0-0.2, 0.2-0.4, and 0.4-0.6 m of soils under residual fertilization from application of sewage sludge (SS) and mineral fertilizers (MF) before cultivation with cowpea.

Attributes	Soil layers					
	0-0.2 m		0.2-0.4 m		0.4-0.6 m	
	SS	MF	SS	MF	SS	MF
pH (H_2O)	4.69	4.70	4.61	4.70	4.96	5.06
[1]TOC (g kg^{-1})	26.40	12.30	12.40	10.30	7.20	6.87
[2]P (mg dm^{-3})	11.18	2.16	3.62	2.26	0.40	0.85
[2]K (mg dm^{-3})	240.00	269.40	211.20	198.80	140.90	141.80
[3]Ca (cmol$_c$ dm^{-3})	2.28	1.63	2.11	1.61	1.93	1.84
[3]Mg (cmol$_c$ dm^{-3})	1.20	1.30	1.07	1.01	0.84	0.92
[2]Fe (mg dm^{-3})	125.70	65.10	89.90	65.80	69.70	55.90
[2]Cu (mg dm^{-3})	1.19	0.90	0.77	0.90	0.87	0.76
[2]Zn (mg dm^{-3})	6.80	0.91	3.53	0.91	1.17	1.65
[2]Mn (mgdm^{-3})	65.20	51.80	51.80	41.10	51.00	59.10
[4]SB (cmol$_c$ dm^{-3})	4.09	3.62	3.72	3.13	3.19	3.21
[5]CEC (cmol$_c$dm^{-3})	5.84	5.18	5.15	4.54	4.67	4.57
[6]BS (%)	70.80	70.00	72.20	68.00	69.90	70.60
[7]SD (g cm^{-3})	1.58	1.67	1.62	1.65	1.65	1.66

[1]total organic carbon, oxidation wet basis with external heating; [2]extracted by Mehlich-1; [3]extracted by KCl 1 mol L^{-1}; [4]Sumof bases; [5]cation exchange capacity at pH 7; [6]base saturation; [7]soil density by the paraffin-coated clod method (DONAGEMA, 2011).

The cowpea crop was irrigated with a micro-sprinkler system, using emitters with mean flow rate of 58 L h^{-1} (equivalent to 5.2 mm h^{-1}), irrigation efficiency of 94%, and a two-day irrigation shift, programed weekly up to the pod maturation stage, with the aid of the Irriplus® (2008) program, based on data from a meteorological station at the experimental area. The crop evapotranspiration (ET_c) was estimated from the reference evapotranspiration (ET_0) obtained by the Hargreaves-Samani model and multiplied by the crop coefficient (K_c) over the crop cycle.

Manual weeding was done at 15, 30, and 45 DAE. Insect control was done as needed from seeding to harvest ,according to Andrade Júnior et al. (2016), with the aid of a database of approved pesticides by the Brazilian Ministry of Agriculture for cowpea crops (AGROFIT, 2016). The cowpea plants were manual harvested at the physiological maturity of each cultivar.

The number of totally expanded and photosynthetically active leaves per plant (NLP) in 20 plants in the evaluation area of each plot were counted at the beginning of the cowpea flowering. Twenty plants per plot were sampled at harvest (approximately 90 days after sowing) for evaluations of root length (RL)from the ground level to the base of the main root; stem base diameter (SD), measured in the lower third of the stem; pod length (PL); 100-grain weight (100GW), using the average of three samples of 100 grains; grain index (GI), by dividing the grain weight of the plot by its pod weight; number of pods per plant (NPP), by counting the pods and dividing by 20 (plants in the evaluation area); grain yield (GY), estimating the total production of the evaluation area of the plot in kg ha^{-1}. The grain moisture was corrected to 13% for the 100GW and GY.

The microbiological analysis was done in 25 g of grains and 25 g of pods of each plot, using plants at pod filling stage (60 DAE) and, subsequently, plants at maturation stage. Samples of pods from the basal, median, and apex regions of branches were collected with sterilized gloves and joint in a single sample for microbiological analysis; the grains were separated from the pods and these samples were placed separately in sterile plastic bags for subsequent analysis.

These sampled grains and pods were evaluated for coliforms at 35 °C and 45 °C (thermo tolerant microorganisms) by the most probable number (MPN) (SILVA et al., 2010). The presence of coliforms at 45 °C was confirmed by inoculation in a *Escherichia coli* broth, using positive test tubes for the analysis of total coliforms, with incubation at selective temperature of 45 °C for 48 hours.

The data were subjected to analysis of variance by the F test, using the Sisvar® program (FERREIRA, 2011) and, when significant ($p \leq 0.05$), the means were compared by the Tukey's test, considering the F test as conclusive for the sources of fertilization. The results related to microbiological evaluation were subjected to descriptive analysis.

RESULTS AND DISCUSSION

The root length (RL) and grain yield (GY) of the cowpea plants evaluated were significantly affected ($p \leq 0.05$) by the isolated factors-cultivars and fertilization (Table 4). The morphological characteristics stem base diameter (SD)and number of leaves per plant (NLP), and the components of production pod length (PL), 100-grain weight (100GW), grain index (GI), and number of pods per plant (NPP) were affected only by the factor cultivar (Table 4). The interaction between the factors was not significant ($p>0.05$) for the evaluated characteristics of the cowpea plants.

Table 4. Analysis of variance, with mean squares (MS) for root length (RL), stem base diameter (SD),number of leaves per plant (NLP), pod length (PL), grain index (GI), 100-grain weight (100GW), number of pods per plant (NPP), and grain yield (GY)of cowpea plants of five cultivars after fertilization with sewage sludge and mineral fertilizers.

Source of variation	DF	MS							
		RL	SD	NLP	PL	GI	100GW	NPP	GY
		cm	cm		Cm	%	g		kg ha^{-1}
Block	3	25.2^{ns}	12.1^{ns}	4129.2^{*}	8.6^{ns}	7.5^{ns}	0.1^{ns}	114.4^{ns}	659728.8^{ns}
Cultivar (C)	4	1201.6^{**}	463.8^{**}	53528.0^{*}	1120.8^{**}	168.9^{**}	61.1^{**}	372.2^{*}	3870008.0^{**}
Fertilization (F)	1	518.2^{*}	19.5^{ns}	59.4^{ns}	5.9^{ns}	0.1^{ns}	2.2^{ns}	18.0^{ns}	3879857.2^{**}
C ×F	4	11.3^{ns}	29.0^{ns}	4100.3^{ns}	1.6^{ns}	13.9^{ns}	0.4^{ns}	31.3^{ns}	25232.5^{ns}
Residue	27	5.9	13.4	1407.2	2.9	14.3	1.0	15.4	222488.1
Mean		24.8	1.6	167.0	21.6	79.7	19.8	16.0	3542.2
CV (%)		9.8	23.0	22.5	8.0	4.7	5.0	25.4	13.3

DF = degrees of freedom; **, *, and ns = significant at 1%, at 5%, and not significant by the F test, respectively. CV =coefficient of variation.

The cowpea cultivars BRS-Pujante and BRS-Cauame presented the highest and lowest RL, respectively (Table 5). The cultivar BRS-Cauamehad the lowest SD (1.32 cm); the other cultivars had similar SD (1.61to 1.72 cm). BRS-Xiquexique, BRS -Pujante, and BRS-Pajeu presented the highest NLP, and BRS-Xiquexique stood out with 183 leaves per plant.

Table 5. Root length (RL), stem base diameter (SD), number of leaves per plant (NLP), pod length (PL), grain index (GI), 100-grain weight (100GW), number of pods per plant (NPP), and grain yield (GY)of cowpea plants of five cultivars.

Cultivar	RL	SD	NLP	PL	GI	100GW	NPP	GY
	cm	cm		cm	%	g		kg ha^{-1}
BRS-Pujante	27.48a	1.71a	180.00ab	25.70a	77.60b	24.60a	18.00a	4124.40a
BRS-Xiquexique	25.77b	1.64a	183.00a	22.20b	79.90b	18.50bc	17.00b	3917.20b
BRS-Pajeu	25.30b	1.61a	178.00b	21.00c	77.70b	17.90c	17.00b	3672.20b
BRS-Marataoa	25.43b	1.72a	146.00c	20.40d	75.80b	19.50b	16.00c	3649.70b
BRS-Cauame	20.19c	1.32b	149.00c	18.60e	87.50a	18.40bc	14.00c	2347.60b

Means followed by the same letter in the columns are not different by the Tukey's test at 5% significance.

The cultivar BRS-Pujante had the highest means for the morphological characteristics evaluated. The highest root length probably affected the other morphological characteristics of this cultivar, resulting in thick stem and high number of leaves. A more vigorous root system is related to plants with higher water and nutrient absorption capacity (VALADÃO et al., 2015), which increases their photoassimilates production, growth, and development. According to Santos (2011), the cultivar BRS-Pujante is recommended for crop sunder irrigation in the São Francisco Valley, especially in the second half of the year, because it has good productive characteristics, such as large grains and pods.

The highest means for the production components PL (25.7 cm), 100GW (24.6 g), and NPP (18 pods plant^{-1}) were found for BRS-Pujante (Table 5). These production components contributed to its higher grain yield. Its better results for production components are attributed to its higher genetic potential, as described by Santos (2011), who reported that this cultivar presented mean PL of 18.4 cm, and 100GW of 24.8 g. These results were lower than those found for the cultivar in the present study, which can be related to the use of irrigation and to the residual effects of fertilizations made for the previous crop. Santana et al. (2019) pointed out the genetic divergences between cowpea genotypes and that the pod weight, flowering beginning time, PL, 100GW, and NPP were the characteristics that contributed the most to genetic divergences.

The other cultivars evaluated in the presented study showed similar GY, from 2,347.62 to 3,917.15 kg ha^{-1} (Table 5). These grain yields were higher than the Brazilian national mean (563 kg ha^{-1}) in the 2017/2018 crop season (CONAB, 2018). The climatic conditions of Janaúba, especially the high temperatures (Figure 1), combined with the balanced irrigation and fertilization throughout the cowpea crop cycle favored the crop performance. These results indicate a good adaptation of the evaluated genotypes to the climatic conditions of the North of Minas Gerais, as described by Silva et al. (2018c). According to Souza et al. (2018), several cowpea cultivars and lines that present satisfactory grain yields and good characteristics of size, lodging, and crop value were recommended for this region of Minas Gerais (lines MNC04-769F-30, MNC05-795F -154, and MNC04-769F-49; and cultivars BRS-Tumucumaque, BRS-Guariba, BRS-Novaera, BRS-Itaim, and BRS-Cauame).

The assays conducted for the register of the cultivar BRS-Pujante indicated grain yields of 705.0 kg ha^{-1} (under rainfed conditions) and 1979 kg ha^{-1} (under irrigation conditions), but without fertilizer applications (SANTOS, 2011). Thus, the use of irrigation combined with mineral fertilization (MF) and residual fertilization from sewage sludge application (SS) shows that the potential grain yield of cowpea is higher in intensive crop systems.

The mean grain yield of the evaluated cultivars was approximately 6.3 times higher than the national mean cowpea grain yield, whose main limitation is the low technological level usually applied to the crop, which is commonly used for subsistence of family farmers (MONTEIRO et al., 2012). Several studies present lower grain yields to those found in Janaúba. This is found for the cultivars BRS-Xiquexique (MATOSO et al., 2013), BRS-Cauame (SANTOS et al., 2016), BRS-Marataoa (SILVA; NEVES, 2011), and BRS-Pajeu (LOCATELLI et al., 2014).

The treatments with residual fertilization from application of sewage sludge presented higher RL than those with mineral fertilization (Table 6), probably due to a higher organic matter content and lower soil density in the 0-20 cm layer (Table 3), which was a favored environment for root development. Thus, higher cowpea GY was found with sewage sludge residual fertilization, with mean of 3,853.7 kg ha^{-1}, which was 19% higher than those found in plants under only mineral fertilization, representing an increase of 10.4 60-kilo bags of cowpea grains per hectare (Table 6).

Table 6. Root length (RL), stem base diameter (SD), number of leaves per plant (NLP), pod length(PL), grain index (GI), 100-grain weight (100GW), number of pods per plant (NPP), and grain yield (GY) of cowpea plants after fertilization with sewage sludge and mineral fertilizers.

Fertilization	RL	GY
	cm	kg ha^{-1}
Sewage sludge	25.64a	3853.70a
Mineral fertilizers	24.03b	3230.80b

Means followed by the same letter in the columns are not different by the F test at 5 %significance.

Applications of sewage sludge to agricultural soils have provided a positive residual effect in the production of crops. Residual fertilization of sewage sludge in wheat crops after rice crops provided 68% higher GY, with the application of 30 Mg ha^{-1}resulting in similar GY to those found in the treatment with NPK mineral fertilization (LATARE et al., 2014). Lobo, Grassi Filho and Kummer (2014) found positive residual effects of sewage sludge fertilization in GY of wheat in the second year of growing, after application of approximately 44 Mg $ha^{-1}$of sewage sludge. The use of organic fertilization combined with irrigation resulted in 54% higher GY (1,422.5 kg ha^{-1}) for cowpea plants of the cultivar BRS-Pujante intercropped with maize, when compared to the control treatment (BRITO et al., 2012). Soil fertilization with sewage sludge presents longer organic compound mineralization and nutrient availability, resulting in residual effects for several crop cycles, reducing production costs (ALBUQUERQUE et al., 2015). It also increases the soil microbial and enzymatic activity, as found by Siebielec, Siebielec and Lipski (2018), who reported that sewage sludge applications over six years favored the enzymatic activity (phosphatases and dehydrogenases), which was stimulated by the higher number of microorganisms in the soil, resulting in higher grain yields for maize, wheat, and barley crops when compared to crops under only NPK fertilization.

The highest GY found for the cultivars under residual fertilization of sewage sludge was attributed to the improvement of soil chemical and physical characteristics, mainly by the increase in organic matter and decrease in soil density (Table 3), which contribute for higher availability of nutrients, especially nitrogen, since this element limits cowpea grain yield because it is the most extracted nutrient by the crop (TAGLIAFERRE et al., 2013). The nitrogen in the sewage sludge is predominantly organic (PIRES et al., 2015), and is gradually mineralized over time and made available to plants (ZARE; RONAGHI, 2019). Thus, nitrogen can be kept longer in soils fertilized with sewage sludge than in soils fertilized with high-solubility fertilizers, which results in increases in grain yield.

No contamination by total coliforms (MPN $g^{-1} < 3$) and thermotolerant microorganisms (MPN $g^{-1} < 3$) was found in fresh grains of any plants at pod filling stage (60 days after sowing) (Table7). However, contamination of fresh pods was found in plants of the cultivars BRS-Pujante, BRS-Cauame, and BRS-Pajeu in the treatments with SS, and in plants of BRS-Cauame, BRS-Xiquexique and BRS-Pajeu in the treatments with MF (Table7). Therefore, contamination was found in cowpea fresh pods in the treatments with SS and MF.

The means of total and thermotolerant coliforms found in fresh pods varied from < 3 to > 1,000 MPN g^{-1}in plants in the treatments with SS, and < 3to 11 MPN g^{-1}in plants in treatments with MF. This contamination was attributed to the semi-prostate growth habit of plants of the cultivars BRS-Pujante, BRS-Pajeu, and BRS-Xiquexique (Table 2), which allowed the fresh pods in the lower parts of the plant to have contact with the soil. These results also indicate the possibility of contamination of humans by the handling of fresh pods, which may be a risk to human health, denoting the need for use of personal protection equipment during the harvest and for microbiological cleaning of fresh pods for human consumption. However, this contamination did not occur internally and did not affect the grains.

The BRS-Cauame cultivar, which has semi-erect growth habit (Table 2) also presented contamination in fresh pods (Table 7). This was probably due to the presence of animals in the experimental area, especially birds and insects, which were frequently seen in the site and over the plant pods.

Table 7. Microbiological analysis of fresh and dry pods and grains of plants of five cowpea cultivars at 60 and 90 days after emergence (pod filling stage and maturation stages, respectivelly), after fertilization with sewage sludge and mineral fertilizers.

Cultivar	Sewage sludge		Mineral		Sewage sludge		Mineral	
	TC	TTC	TC	TTC	TC	TTC	TC	TTC
	MPN g^{-1}							
	60 days after emergence (pod filling stage)							
	Fresh grain		Fresh grain		Fresh pod		Fresh pod	
BRS-Pujante	<3.0	<3.0	<3.0	<3.0	>1100.0	>1100.0	<3.0	<3.0
BRS-Marataoa	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0
BRS-Cauame	<3.0	<3.0	<3.0	<3.0	>1100.0	290.0	6.1	<3.0
BRS-Xiquexique	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	11.0	11.0
BRS-Pajeu	<3.0	<3.0	<3.0	<3.0	9.0	<3.0	11.0	11.0
	90 days after emergence (maturation stage)							
	Dry grain		Dry grain		Dry pod		Dry pod	
BRS-Pujante	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0
BRS-Marataoa	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0
BRS-Cauame	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0
BRS-Xiquexique	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0
BRS-Pajeu	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0
Tolerance	500.0		500.0		500.0		500.0	

MPN = most probable number; TC = total coliforms; TTC = thermotolerant coliforms; Tolerance = maximum tolerance to presence of microorganisms allowed by the resolution RDC nº 12 of 02/01/2001 (BRASIL, 2001).

The results found in the present study confirm those of Alves et al. (2017), who evaluated microbiological characteristics in banana fruits treated with effluents from the same treatment station and found thermotolerant coliforms and *Escherichia coli* in banana peels in the control without application of sewage effluent; they attributed this result to birds and bats frequently found over the banana trees in the experimental area.

The contamination indexes of fresh pods of the cultivars BRS-Marataoa, BRS-Xiquexique, and BRS-Pajeuwere below the tolerance limit (500 MPN g^{-1}) established by the Anvisa (BRASIL, 2001), in all treatments (SS and MF) (Table 7). This was probably due to the plant growth environment, which had high solar radiation and low air humidity and soil moisture during some cowpea growth stages. According to García Orenes et al. (2007), the edaphoclimatic conditions of the semiarid region of the state of Minas Gerais contribute to reduction of coliforms; they evaluated the survival rate of total coliforms in different agricultural soils (with and without irrigation)in this region after incorporation of sewage sludge and found that low air humidity reduces total coliforms in the soil. Different from other bacteria, coliforms present no resistance to absence of irrigation. This denotes that the period with no irrigation (8 months) between the pineapple and cowpea crops contributed to the reduction of coliforms in the soil from the fertilization with sewage sludge.

Fresh cowpea pods are commonly discarded, and the grains are consumed after cooking. Oliveira et al. (2008) evaluated the microbiological quality of cowpea grains and found 430 to 100,000 MPN g^{-1} of total coliforms in raw grains, and 10 MPN g^{-1} of total coliforms in cooked grains, with or without water at maceration, denoting that the thermal treatment reduced the total coliforms in raw samples.

After the cowpea pod maturation (90 DAE), the contamination by total coliforms (MPN $g^{-1} < 3$) and thermotolerant microorganisms (MPN $g^{-1} < 3$) was null in grains and pods of plants in all evaluated treatments (Table 7).

The survival of pathogenic microorganisms in contact with plants depends on environmental factors, because air temperature, solar radiation, and air humidity conditions are commonly unfavored to the pathogen. According to Jang et al. (2017), the survival of microorganisms, including *Escherichia coli*, is low when they are exposed to abiotic factors, such as high air temperatures (>30 C),high solar radiation, and low air humidity. The harvest of dry pods occurred at more than 10 days after the irrigation was stopped, which probably hindered the presence of pathogenic microorganisms that needed water for their growth and metabolism. Moreover, the edaphoclimatic conditions of the semiarid region of the state of Minas Gerais may have contributed to the reduction of coliforms in the soil.

Currently, the consumers are more concerning about the nutritional value of foods, and absence of toxic elements and microbiological contaminants in them. The present work showed that a food with adequate quality for human consumption was obtained, according to the standards established by the Anvisa (BRASIL, 2001), regardless of the time when the analyses were carried out-at pod filling (fresh grains) and maturation (dry grains) stages.

CONCLUSIONS

Cowpea plants can be grown in areas with residual fertilization of sewage sludge, which increases their growth and yield. Considering irrigated crops, the cultivar BRS-Pujante is the most productive cowpea cultivar, followed by BRS-Xiquexique, BRS-Pajeu, and BRS-Marataoa, which present intermediate values for production components; the cultivar BRS-Cauame has lower values for production and vegetative components than the other four cultivars. The residual fertilization of sewage sludge resulted in cowpea fresh and dry grains with similar microbiological quality to those under mineral fertilization, but requiring higher are for human consumption of fresh pods of plants grown in soils that have previous soil fertilization with sewage sludge.

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