



# Biochemical changes and development of soybean with use of pelletized organomineral fertilizer containing sewage sludge and filter cake

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**ABSTRACT.** Filter cake has been one of the most widely used waste products as a source of organic matter in the production of biofertilizers. However, sanitized sewage sludge is a recommended alternative for agricultural use because of environmental issues. Studies that examine the use of this particular class of fertilizer are needed. The objective of this work was to evaluate the efficiency of sewage sludge and filter cake as sources of organic matter in the composition of pelletized biofertilizers for soybean production. The experimental design was a randomized block design in a 2 x 4 + 2 factorial, which includes two sources of organic matter (sanitized sewage sludge and filter cake) and four levels of nitrogen (50, 75, 100, and 125%) relative to the recommended dose of phosphorus pentoxide compared with the mineral fertilizer. The use of organomineral fertilizer with the formulation of 75% sewage sludge showed greater growth for aerial plant parts. Biofertilizers formulated with sewage sludge and filter cake can replace mineral fertilizer and increase soybean growth. Quantitative changes in peroxidase, catalase and urease activity, as well as lipid peroxidation, were observed following the use of biofertilizers.

**Keywords:** fertilization; antioxidant enzymes; organic matter.

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## Introduction

Cultivation of soybean (*Glycine max* (L.) Merrill.) is important worldwide, with Brazil being the second largest producer of this grain behind the United States (Conab, 2017). Modern soybean production uses the most advanced inputs and equipment. Biofertilizers, in the form of pellets, have great potential as an input that adds technology and countless possibilities for soybean production.

Organomineral fertilizers are an alternative to fertilizers formulated exclusively with mineral sources. For example, filter cakes have the advantage of environmental safety because they are composed of productive chains that were not previously designated as waste and residues of agribusiness (Kiehl, 2008; Cardoso, Lana, Soares, Peixoto, & Luz, 2017; Oliveira et al., 2017).

Sewage sludge is a residue rich in organic matter that is generated during the treatment of wastewater and sewage. The process of treatment aims at separating the solid part of the liquid such that the treated effluent can return to water reservoirs without causing damage to the environment (Corrêa, Fonseca, & Corrêa, 2007). In this process, the concentrated sludge contains organic matter and nutrients and is considered a byproduct of treatment (Bettiol & Camargo, 2006). When this residue is treated for subsequent use in agriculture as a fertilizer, it is referred to as BIOSOLIDS. This term was created by the Water Environmental 10 Federation (WEF) with the primary objective of minimizing rejection and increasing the dissemination of knowledge about the benefits of this material (Higashikawa, Silva, & Bettiol, 2010).

According to Bueno et al. (2011), sewage sludge or BIOSOLIDS typically contains approximately 40% organic matter, 4% nitrogen, and 2% phosphorus. The other macronutrients and micronutrients are found in smaller quantities, except for biosolids sanitized by liming, where higher concentrations of calcium and magnesium are added.

Filter cake is created from the process of treatment and clarification of sugarcane juice. Filter cake has been one of the main wastes employed in the manufacture of pelletized biofertilizers. Filter cake can be

used as a reference for new sources of organic matter due to its chemical, physical, and microbiological characteristics that are desirable for the manufacture of fertilizers. Filter cake can be applied over the total area, during preplanting, in the groove or between the lines of planting and is rich in organic matter, macro and micronutrients; in 20 t ha<sup>-1</sup> of filter cake, it is possible to provide 100% nitrogen, 50% phosphorus, 15% potassium, 100% calcium, and 5% magnesium to sugarcane (Fravet, Soares, Lana, Lana, & Korndörfer, 2010). Research related to the use of sewage sludge in the formulation of biofertilizers is still limited, especially pertaining to the formation of pellets.

Teixeira, Sousa, and Korndörfer (2014) concluded that organomineral fertilizer is a viable alternative for total or partial substitution of conventional mineral fertilization, and it provides results similar or superior for growth traits of sugarcane plants. Resende Júnior, Camargo, Lana, Alves Filho, and Matos (2016) affirm that the biosolids present themselves as an alternative for complementary soil fertilization for cultivation of *Brachiaria brizantha* cv. Marandu, highlighting its potential as a soil amendment. Camargo, Maldonado, Dias, Souza and França (2013) used biosolids in the substrate for production of *Jatropha* seedlings (*Jatropha curcas* L.) and observed the elevation of nitrogen, sulfur and leaf micronutrient levels. According to Oliveira et al. (2017), the biofertilizers formulated with sanitized sewage sludge, peat or filter cake are capable of replacing the mineral fertilization for sorghum production, even with a reduction in the dose applied relative to mineral fertilizer.

The objective was to evaluate the efficiency of pelletized biofertilizers formulated from sanitized sewage sludge and filter cake for soybean production by means of phenometrics and biochemical evaluations.

## Material and methods

The study was conducted in a greenhouse located in the University Center of Patos de Minas, in the municipality of Patos de Minas, Minas Gerais State, Brazil. The site resides at the geographic coordinates 18°57'28" S (latitude), 46°51'31" W (longitude) and 815 m of altitude. The soil used was classified as an Oxisol. Before treatment, a chemical analysis test was performed on a sample of soil, and the results are presented in Table 1.

**Table 1.** Chemical characterization of a soil sample collected from Patos de Minas, Minas Gerais State, Brazil, in 2017.

Textural class	pH H <sub>2</sub> O	O.M.	TOC	P	K	Zn	Ca	Mg	Al	H+Al	SB	CTC (t)	CEC (T)	m	BS
		-- g kg <sup>-1</sup> --		----- mg dm <sup>-3</sup> -----						cmolc dm <sup>-3</sup>				----- % -----	
Clay	5.45	39.9	23.1	10,14	189	9.7	2.13	1.15	0.06	3.62	3.76	3.82	7.38	1.57	50.99

\*O.M.: Organic Matter; TOC: Total Organic Carbon; SB: Sum of Bases; CEC: Cation Exchange Capacity; m%: Aluminum Saturation; BS%: Base Saturation (Donagema, Campos, Calderano, Teixeira, & Viana, 2011).

The experiment was conducted using seeds of soybean (*Glycine max* L. Merrill); the variety grown was NA 5909 RG from maturation group 6.7. Before sowing, the seeds were treated with: Standak Top<sup>®</sup> (fungicide + insecticide), which contains Fipronil (250 g L<sup>-1</sup>), Thiophanate Methyl (225 g L<sup>-1</sup>) and pyraclostrobin (25 g L<sup>-1</sup>) at a dose of 2 mL kg<sup>-1</sup> of seeds; and CoMo Platinum<sup>®</sup> composed of molybdenum (235.5 g L<sup>-1</sup>), cobalt (23.55 g L<sup>-1</sup>), and phosphorus (43.96 g L<sup>-1</sup>) at a dose of 1.5 mL kg<sup>-1</sup> of seeds. The inoculation of seeds was made using Azofix<sup>®</sup> (4 mL kg<sup>-1</sup>) and 10 seeds were sown per pot to a depth of 3.0 cm. Thinning to seven plants per pot of 10 L was performed 10 days after emergence.

In the experiment, two biofertilizers, having sanitized sewage sludge (biosolids) or filter cake organic bases, were used in the formulation of pellets containing NPK 03-17-10 fertilizer treatment (100% of the recommended dose), produced by the company Geociclo Biotecnologia S.A. Mineral. The same sources were used in the production of organomineral fertilizer (urea, triple superphosphate and potassium chloride).

For the production of organomineral fertilizer based on filter cake, approximately 60 kg of urea, 370 kg of triple superphosphate, 165 kg of potassium chloride, and 405 kg of filter cake were mixed. For the production of the organomineral fertilizer based on sewage sludge approximately 65 kg of urea, 370 kg of triple superphosphate, 165 kg of potassium chloride, and 400 kg of sewage sludge were mixed.

The sanitized sewage sludge used in the study was obtained from the effluent treatment station in the municipality of Uberlândia, Minas Gerais State, Brazil. The sanitization of the residue, aiming at the elimination of pathogens and the reduction of moisture, was done according to the methodology proposed by Alves Filho et al. (2016). Filter cake was collected from the Usina Tijuco Valley, located in the municipality of Uberaba, Minas Gerais State, Brazil. Chemical characterization of filter cake and sewage

sludge prior to sanitizing was performed at the Laboratory of Analysis of soils from the Federal University of Uberlândia (Table 2).

**Table 2.** Chemical characteristics of sewage sludge and filter cake used for formulation of biofertilizers. Patos de Minas, Minas Gerais State, Brazil, in 2017.

	pH H <sub>2</sub> O	O.M.	N total	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	C/N ratio
				----- % -----		
Sewage sludge	7.60	19.80	0.79	2.23	0.24	28/1
Filter cake	6.81	23.49	0.61	0.95	0.30	13.7/1

\*K<sub>2</sub>O: Potassium Oxide; O.M: Organic Matter.

The experimental design was a randomized block in a 2 x 4 +2 factorial. Two pelletized biofertilizers with sanitized sewage sludge and filter cake organic basis were treated with four levels of phosphorus (50, 75, 100, and 125%) relative to the rate of phosphorus pentoxide (P<sub>2</sub>O<sub>5</sub>) recommended by the Commission of Soil Fertility in the State of Minas Gerais (Ribeiro, Guimarães & Alvarez, 1999), and two additional treatments (mineral fertilization and absence of fertilization), as shown in Table 3.

**Table 3.** Organomineral fertilizers (FOM), rates and doses of nitrogen, phosphorus pentoxide and potassium oxide. Patos de Minas, Minas Gerais State, Brazil, in 2017.

Treatment	Fertilizer %	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
		----- mg kg <sup>-1</sup> -----		
Without fertilization	-	-	-	-
FOM (Sewage Sludge- SS)	50	5.29	30	17.64
FOM (Sewage Sludge- SS)	75	7.94	45	27.47
FOM (Sewage Sludge- SS)	100	10.58	60	35.28
FOM (Sewage Sludge- SS)	125	13.23	75	44.10
FOM (Filter Cake- FC)	50	5.29	30	17.64
FOM (Filter Cake- FC)	75	7.94	45	27.47
FOM (Filter Cake- FC)	100	10.58	60	35.28
FOM (Filter Cake- FC)	125	13.23	75	44.10
Mineral Fertilizer	100	10.58	60	35.28

- absence of nutrient.

The application of fertilizers was performed at 4.0 cm depth and adjacent to the seed. During the experiment, the pots were irrigated daily according to the need for water (400 mL per 10 L pot until the end of the pod-filling phase).

The evaluation of root dry mass (RDM) was performed 30 days after seeding, whereas the dry mass of stems (DMS) and leaves (DML) were determined at 30, 60, and 80 days. Two plants per experimental unit were used to perform the analyzes. At each time of collection, plant roots, stems, leaves and pods were separated, then wrapped in individual paper bags, and dried using an oven with forced air circulation at a temperature of 65°C until constant weight.

For biochemical measurements, completely expanded leaves from the middle third of the plants at 45 DAS (days after sowing) were collected and transported in Styrofoam boxes containing ice. The material was used for all biochemical determinations. Extraction of the plant material used for determination of total protein and catalase, superoxide dismutase, and peroxidase enzymes was performed according to the methodology proposed by Kar and Mishra (1976).

The superoxide dismutase enzyme was assessed according to the methodology proposed by Beauchamp and Fridovich (1971). Catalase enzyme activity was determined by using the method described by Peixoto, Cambraia, Santanna, Mosquim, and Moreira (1999). The activity of the peroxidase enzyme was determined in accordance with the conditions cited in the work of Teisseire and Guy (2000). The activity of the enzyme urease was performed according to the methodology proposed by McCullough (1967).

The total soluble protein content in the leaves was performed according to the methodology described by Bradford (1976). The content of H<sub>2</sub>O<sub>2</sub> inside the cells was determined by the method of indication made by the reaction with potassium iodide (KI), as reported by Alexieva, Sergiev, Mapelli, and Karanov (2001). The quantification of lipid peroxidation in cell membranes was determined in accordance with the technique reported by Heath and Packer (1968).

The data obtained in the experiment were subjected to tests of presuppositions (Levene's test and Shapiro Wilk). Tukey test (at 5% significance level) was used to compare the treatments using the SISVAR program (Ferreira, 2011). The Dunnet's test was done to compare the biofertilizers with and without mineral fertilization. The study of biofertilizer rates was performed by regression to obtain the statistical models.

## Results and discussion

At 30 DAS the analysis of the weight of raw mass of roots revealed that the organomineral fertilizer with sewage sludge at rates of 100 and 125% (relative to the recommended dosage) and with filter cake at rates of 50 and 75% were superior compared with mineral fertilizer (Table 4). The influence of biofertilizers on root growth are widely discussed in the literature. Increases in yield of tubers of potato were observed when organomineral fertilizer derived from chicken bedding was used (Cardoso, Luz, & Lana, 2015). The greater root growth in plants subjected to the application of biofertilizers may be related to the presence of amino acids and humic substances. Studies have suggested that humic substances can stimulate the growth of the plant in terms of increased stature and the accumulation of dry mass; these effects depend upon the source and concentration of humic substances used (Elgala, Metwally, & Khalil, 1978).

The structure of humic substances has empty spaces of different sizes, allowing hydrophobic and hydrophilic organic compounds, such as carbohydrates, lipids, proteinaceous substances, pesticides and other pollutants, as well as inorganic elements, to become integrated within this structure (Albers, Banta, Hansen, & Jacobsen, 2009; Clarholm, Skyllberg, & Rosling, 2015)

The humic acid has important physiological characteristics, such as the ability to: improve the absorption of nutrients by the root system; optimize and initiate the effect of auxin within and outside of the plant; and act in cellular respiration and in the synthesis of proteins and enzymes. The increase in absorption occurs because of a stimulation of proton pumps that form an electrochemical gradient.

The use of biofertilizers increases nutrient use efficiency by activating enzymes that stimulate the absorption and the chelation of some nutrients; they provide growth-promoting organic molecules and promptly modify the characteristics of the soil.

**Table 4.** Dry mass weight of roots (DWR) at 30 days and dry mass of stems (DMS) and dry mass of leaves (DML) at 30, 60 and 80 days after sowing, according to sources of organic matter in the composition of biofertilizers, Patos de Minas, Minas Gerais State, Brazil, in 2017.

Treatment	DWR		DMS		DML		
	30 DAS	30 DAS	60 DAS	80 DAS	30 DAS	60 DAS	80 DAS
WF	2.87	4.14	11.40	11.00	4.56	8.97	7.36
SS 50%	3.43	4.26	12.45	14.21°	4.68	10.13	10.67**
SS 75%	3.51	5.66**	13.09*	17.38°	5.47*	11.34**	11.94**
SS 100%	4.10**	5.56**	12.24	14.71°	4.75	9.52	11.27**
SS 125%	4.95**	4.33	11.18	11.80°	4.33	8.84°	10.26**
FC 50%	3.76*	5.43**	13.32*	11.77°	5.47*	11.16*	10.48**
FC 75%	5.00**	6.40**	8.95**	12.15°	5.86**	8.30**	11.79**
FC 100%	2.62	5.76**	13.11*	12.45°	5.38	10.82*	12.88**
FC 125%	2.58	5.59**	12.79	12.54°	5.11	9.80	12.02**
MF	3.03	4.22	12.35	8.58	4.60	10.11	7.47
CV (%)	12.93	9.71	6.48	10.18	9.95	5.96	9.52

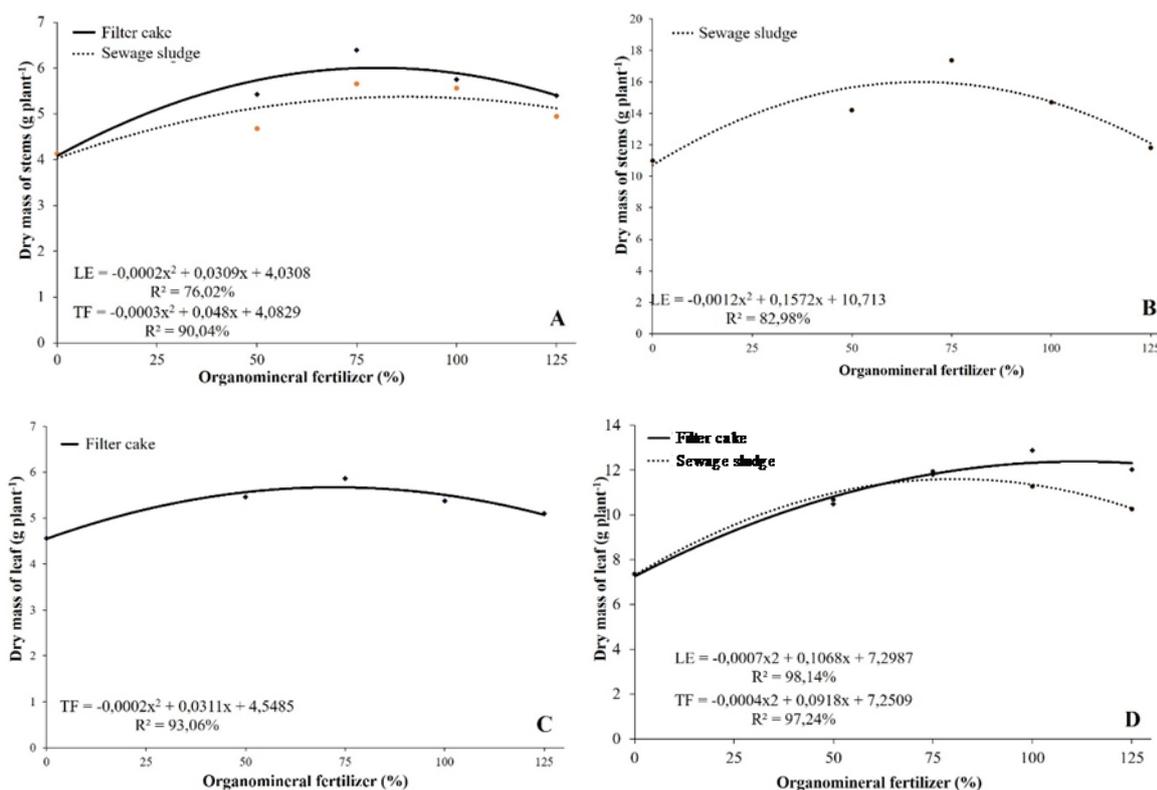
\*Significant difference relative to the treatment without fertilizer (WF) by Dunnet's test at 0.05 significance level. \*\*Significant difference relative to mineral fertilizer (MF) by Dunnet's test at 0.05 significance level.

The assessments at 60 and 80 days were not performed for root dry mass because we observed breakage and loss of roots during collection. According to Cope and Evans (1985), these results can mask the effects of treatments used in tests conducted in the greenhouse when performed in pots.

The 30-day assessments for all rates of the fertilizer organomineral formulated with filter cake resulted in higher stem dry mass relative to the treatments with or without mineral fertilization, whereas for fertilizer formulated with sewage sludge, only the rates of 75% and 100% differed from both the additional treatments. Orlando Júnior, Seidel, Rampim, Neto, and Coppo (2016) reported higher plant growth when compared with mineral fertilizer when the number of pods per plant and number of pods with two or three grains in soybean plants were evaluated following application of organomineral fertilizer.

The growth of aerial structure is typically related to rooting vigor. This relationship is caused by several factors, such as the increase in the number of roots, which allows greater effective area of soil access to absorb water and nutrients. In addition, roots synthesize the hormone cytokinin, which promotes the development of aerial plant organs (Fagan, Ono, Rodrigues, Soares, & Dourado Neto, 2016). Tiritan, Santos, Bordini, Foloni, and Onishi (2010) emphasized that the use of organomineral fertilizer in maize induced an increase in dry mass of the plants compared with mineral fertilizer. The evaluation performed at 30 DAS for DML showed that only the nitrogen fertilizer formulated with filter cake at a dose of 75% (FC 75) managed to be superior to mineral fertilizer, whereas at 80 days the mineral fertilizer was overcome by all biofertilizer treatments (Table 4). It is clear that the effects of the biofertilization relative to mineral fertilization were more expressive until the final evaluation at 80 days. This phenomenon may reflect one of the most important characteristics of the biofertilizers, i.e., a slower release relative to mineral fertilizers, where nutrients are more available until the end of the crop cycle.

In the regression analysis for the variation of DMS as a function of rates of fertilizers assessed at 30 days (Figure 1A), the rates of 77.25 and 80% provided the best values of DMS for the fertilizer composed of sewage sludge and filter cake, respectively. Across the range of rates studied, filter cake provided the greatest increase in dry mass of stems, probably related to other properties associated with this source of organic matter. The filter cake is currently recognized and widely used in the production of organomineral fertilizers.



**Figure 1.** Regression of phenometrics data for soybean plants. (A) Accumulation of dry mass of stems at 30 DAS; (B) Accumulation of dry mass of stems at 80 DAS; (C) Accumulation of dry mass of leaves 30 DAS; and (D) Accumulation of dry mass of leaves 80 DAS.

Figure 1B shows that at 80 DAS, there was a regression curve adjustment only for the source LE and at 70% of the recommended dose there was a greater increase in dry mass of stems.

The use of organomineral fertilizers promoted significant growth of the aerial plant parts when applied to crops such as soybean *Glycine max* (L.) Merrill (Martins et al., 2017), maize (*Zea mays*) (Tiritan et al., 2010), rice (*Oryza sativa*) (Audu & Samuel, 2015) and sugar cane (*Saccharum officinarum*) (Souza, Vitti, Sanquetta, & Gaitarossa, 2016). Alley and Vanlauwe (2009) argue that the growth of the aerial part of a plant is closely related to the development of the roots and both are associated with the fertility of the soil.

The regression analysis presented in Figure 1C demonstrates that for the organomineral fertilizer composed of filter cake, a dose of 80% resulted in a greater increase of DML at 30 days. However, at 80 days (Figure 1D), organomineral fertilizer composed of filter cake resulted in greater DML with a higher dose, close to 115% relative to the recommendation for culture. Similar results were reported by Oliveira et al.

(2017), where biofertilizers showed an increase in dry mass for aerial plant parts relative to mineral fertilizer, particularly for fertilizer composed of filter cake.

The activities of antioxidant enzymes showed different behaviors that depended on the application of the treatments. For activity of superoxide dismutase (SOD) and total soluble protein concentration (TSP), no significant differences were observed between treatments (Table 5). The SOD enzyme typically shows an increase of activity when the plants are subjected to a stressful environment, such as salinity, high temperatures, water stress, high intensity, phytopathogenic and insect attack (Broetto, Lüttge, & Ratajczak, 2002).

**Table 5.** Activity of catalase (CAT), peroxide dismutase (POD), superoxide dismutase (SOD), urease (U), total soluble protein (TSP), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and lipid peroxidation (LP) for soybeans after application of organomineral fertilizers with sewage sludge (SS) and filter cake (FC), Patos de Minas, Minas Gerais State, Brazil, in 2017.

	TSP	SOD	POD	CAT	H <sub>2</sub> O <sub>2</sub>	LP	U
	mg g <sup>-1</sup>	U μg <sup>-1</sup>	μmol min. <sup>-1</sup> μg <sup>-1</sup>	μmol min. <sup>-1</sup> μg <sup>-1</sup>	μmol g <sup>-1</sup>	nmol	μmol
	[MF]	[protein],	[protein],	[protein],	[MF]	[TBARS]	[NH <sub>4</sub> ] g <sup>-1</sup> [MF] h <sup>-1</sup>
Control	9.86	13.22	1.02	3.13	11.96	68.49	8.63
SS 50	11.50	15.75	0.94	4.97°	12.46	74.47	9.76
SS 75	10.08	18.53	1.12	6.39*°	11.92	66.60	9.76
SS 100	9.82	25.88	1.59	4.52°	13.54	67.62	12.29*
SS 125	10.69	20.76	1.23	3.86	13.46	66.09	11.38
FC 50	11.11	20.49	0.78	5.92*°	12.14	64.93	11.26
FC 75	9.85	20.73	1.10	2.41	11.45	69.85	9.48
FC 100	9.51	19.54	1.03	5.12°	12.95	74.41	10.34
FC 125	12.97	15.66	0.57°	1.26	11.98	77.62°	11.72*
Control	8.80	19.76	1.34	1.92	13.07	63.31	10.39
CV (%)	23.22	36.87	31.03	26.09	12.27	8.40	13.32

\*Significant difference relative to the treatment without fertilizer (SF) by Dunnet's test at 0.05 significance level. °Significant difference relative to mineral fertilizer (MF) by Dunnet's test at 0.05 significance level.

Compared with peroxidase (POD) there was no significant difference for the two sources used, except for the treatment FC 125, which showed a reduction of 78% and 135% relative to the treatment without and with mineral fertilization, respectively. Conversely, Garcia et al. (2014) used organomineral fertilizer in the cultivation of rice, and observed that there was greater POD activity resulting in a reduction of H<sub>2</sub>O<sub>2</sub> content and greater conservation of membrane permeability, which was not consistent with results from this study. The enzyme activity of catalase (CAT) was high in the treatments (50 for filter cake and 75 for sewage sludge), which was different from treatments with and without mineral fertilization. Carneiro et al. (2015), evaluated the effects of different nitrogen sources on antioxidative metabolism in rubber plants and verified that treatments which received higher concentrations of nitrogen showed higher activities of CAT. In work with sunflowers there were significant increases in CAT activity due to the application of N through organic fertilizer which originated from the decomposition of waste (Nunes Junior et al., 2017).

The quantification of antioxidant enzyme activity has been performed widely to analyze the oxidative stress in plants due to various biotic and abiotic factors. Vasconcelos, Zhang, Ervin, and Kiehl (2009), applied biostimulation to soybean cultivars and found an increase in the activity of SOD, CAT and POD enzymes. Balakhnina and Borkowska (2013) affirm that plants susceptible to environmental stressors presented higher enzymatic activity of SOD, CAT and POD as a response to the increase in the levels of free radicals inside the cells. No significant difference was observed for the content of H<sub>2</sub>O<sub>2</sub> between the treatments.

The results found for lipid peroxidation (LP) indicate that the dose of 125% of organomineral fertilizer containing filter cake provided an increase of 13% and 22% compared to the treatment without and with mineral fertilization, respectively. The enzyme activity of POD for this treatment was significantly reduced, which could be explained by the high content of lipid peroxidation that occurred in this treatment. Plants have several lines of defense against oxidative stress. In the case of treatment with FC 125%, it is possible that the application of this fertilizer influenced the ability of the plant to metabolize the enzymes responsible for the elimination of reactive species of oxygen (RSOs). According to Gill and Tuteja (2010), the enzymatic and nonenzymatic metabolisms are not able to degrade the free radicals produced during the lipid peroxidation of membranes, which may result in the death of the cell.

With regard to the activity of the enzyme urease, only the sewage sludge 100 and filter cake 125 treatments statistically differed in relation to the treatment without fertilization. The treatments provided an increase of 42 and 36%, respectively. The activity of the enzyme urease plays a major role in the metabolism of ureides, especially in breach of urea, which subsequently results in the formation of  $\text{NH}_3$  that will be used in the formation of amino acids (Fagan et al., 2016).

## Conclusion

The biofertilizers formulated with the organic bases of sanitized sewage sludge and filter cake can replace mineral fertilizer.

The use of biofertilizers based on sewage sludge provides a 25% reduction in the required dose of mineral fertilizer, leading to greater growth increase.

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