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Glycerin effluent from the biodiesel industry as potassium source to fertilize soybean crop

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This research is aimed at evaluating the effects of the glycerin effluent from the biodiesel industry resulting from chemical processes involving the transesterification of triacylglycerides in bio-oils, a reaction catalyzed with potassium hydroxide. This glycerin material (hereinafter referred to as "K-glycerin") may be assumed to be a potential source of potassium to increase the yields of soybean crops and to promote corresponding alterations in some chemical and microbiological properties of the soil. Two field assays were conducted on two soils classified (i) Typical Hapludox (Perox suborder of the Oxisol order, according to the USDA Soil Taxonomy System. In this work, this site was identified simply as TH) and (ii) Typical Quartzipsamment (Aquents suborder of the Entisols order; TQ), in the state of Minas Gerais, Brazil. The used treatments were: (i) four doses (corresponding to 0, 40, 80 and 160 kg ha⁻¹ K₂O) on the TH soil and four doses (corresponding to 0, 60, 120 and 240 kg ha⁻¹ K₂O) on the TQ soil form, and (ii) two potassium sources (specifically, KCl and K₂SO₄) at a K₂O equivalent doses of 80 kg ha⁻¹ on the TH soil and 120 kg ha⁻¹ on the TQ soil. The soybean yields resulting from the application of K-glycerin were found to be related to the availability of K in the soil and this source supplied in part the need of potassium by soybean crop. The K-glycerin does not cause any readily detectable harmful or environmental effect to these cropping sites; however, further detailed studies are needed to better evaluate the long-term use to understand the soil ions dynamics under the soybean crop.

Key words: Organic residue, yield, nutrition, soil, microbial biomass.

INTRODUCTION

The residual crude glycerin from the biodiesel industry corresponds to about 10 to 15% of the total biodiesel mass production. In the lack of any specific legislation indicating how to dispose such residue, much of this by-product is more commonly accumulated in areas of the industrial plants. The expected expansion of the worldwide production of biodiesel will in consequence tend to increase the stock of glycerin (Ooi et al., 2004).
The residual crude glycerin from the biodiesel industry usually contains about 50 mass% of pure glycerin (Zhou et al., 2008; Carvalho et al., 2012) and is rather dark, relatively to the pure glycerin; it needs to be purified for further use in the fine chemistry industry (Yong et al., 2001). However this process is expensive and the effluent is usually discarded as waste glycerin by small industries producing biodiesel (Dasari et al., 2005).

Following the increasing production of biodiesel worldwide, the glycerin waste is thought to be a potential environmental problem although it may also represent an opportunity to get environmental and economical profits depending only on new technological developments favoring its rational use. Many challengeable alternatives may be put under considerations, in attempts to confer better technological uses, reduce the environmental impacts and consolidate biodiesel as an environmentally and economically competitive biofuel (Ito et al., 2005). Some technological uses of crude industrial glycerin as feedstock have been reported (Zhou et al., 2008), although its potential use as soil fertilizer for agricultural lands is still unknown.

Soybean oil is the most widely used for the production of biodiesel (Freedman et al., 1986; Noureddini and Zhu, 1997). Thus the lower the cost of the soybean production the more interesting would be its industrial use. A conceivable alternative for the agricultural use of crude glycerin from the transesterification of triacylglycerides of bio-oils to produce biodiesel, using potassium hydroxide as catalyst, would be to neutralize the effluent with phosphoric or sulfuric acid. The potassium phosphate or sulfate formed during this step would improve the use of the effluent as fertilizer (Zhou et al., 2008).

Potassium is the second most promptly absorbed macronutrient after nitrogen. The plant nutritional requirements may be variable, depending on innumerable conditions of soil and the plant itself. On average, it assumes an uptake of 81 kg N and 54 kg K to produce 1,000 kg of soybean grains (Borket and Yamada, 2000). Some studies have shown that when levels of available potassium in soil are above 60 mg dm$^{-3}$, the yield responses of the soybean plant to potassium fertilization are usually not significant (Scherer, 1998a, b). Maximum yields of soybeans were reportedly attained with application of 60 kg ha$^{-1}$ (Scherer, 1998b); 80 and 120 kg ha$^{-1}$, if the level of available K in soil was between 16 and 40 mg dm$^{-3}$ (low content) and between 41 and 70 mg dm$^{-3}$ (mean content), respectively (Novais, 1999) and 85 and 90 kg ha$^{-1}$ K$_2$O in case of no-tillage land management (Foloni and Rosolem, 2008).

In tropical and subtropical soils, organic matter has a close relationship with other physical, chemical and biological soil properties, chemical among the cation exchange capacity (CEC), is of fundamental importance for maintaining the productive capacity of the soil longer term (Ciotta et al., 2003; Araújo et al., 2007; Moreti et al., 2007; Carvalho et al., 2011). Thus, industrial glycerin, as an organic residue, would tend to increase the usually low CEC of tropical soils, to reduce loss of cations, particularly K$^+$, and improve their fertility.

In this work, it was used sulfuric acid to neutralize the material of the glycerin effluent. The resulting raw product containing potassium sulfate was assayed as fertilizer for soils supporting the growth of soybean plants. The study thus aimed at evaluating the use of the glycerin effluent (the material will be hereinafter referred to as “K-glycerin”), a waste product of the biodiesel industry, from the transesterification of triacylglycerides in a vegetable oil catalyzed with KOH, as a potassium source on the yield and nutrition of soybean plants and on alterations of the chemical and microbiological properties of the soil.

MATERIALS AND METHODS

Description of the studied area

Two experiments were carried from December 2009 to April 2010 at two different soil sites, both in the state of Minas Gerais, Brazil: (i) of a Typical Hapludox (Perox suborder, Oxisol order; TQ), in an area (18° 15’ S 43° 36’ W; altitude of 1,400 m a.s.l.; Köppen climate, Aw, with a mean annual rainfall of 1,200 mm; mean temperature 24°C) located at the Moura Experimental Farm of the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM), in the municipality of Curvelo; (ii) of a Typical Quartzipsamment (Aquents suborder, Entisols order; TQ), in an area (18° 15’ S 43° 36’ W; altitude of 1,400 m a.s.l.; Köppen climate, Cwb; mean annual rainfall of 1,082 mm; mean temperature 19.4 °C) located in the Campus JK UFVJM, in the municipality of Diamantina.

The soil samples were air-dried, sieved (2.0 mm) and characterized according to Embrapa (1997) (Table 1). The pH was potentiometrically measured (soil:water 1:2.5, v/v); phosphorus and potassium were extracted with the Mehlich-1 solution and determined by colorimetry (for phosphorous) and flame photometry (potassium); calcium, magnesium and aluminium were extracted with a solution of 1 mol L$^{-1}$ KCl and determined by flame atomic absorption spectrophotometry (calcium and magnesium) and titrated with 0.025 mol L$^{-1}$ NaOH, for aluminum; the acidity (H + Al) was determined by extracting with 0.5 mol L$^{-1}$ calcium acetate buffered at pH 7.0 and quantified by titration with 0.025 mol L$^{-1}$ NaOH. The organic carbon (OC) was determined by Walkley-Black method (Walkley and Black, 1934). The sulfate (SO$_4^{2-}$) content was determined according Hoeft et al. (1973). Values of cation exchange capacity (CEC), Al saturation (m) and base saturation (V) were then calculated. The pipette method was used to determine the soil texture (Embrapa, 1997).

Planting and agronomic practices

The preparation of the experimental area was made by the
conventional system with disc plow to 0.4 m deep and two disking with disc harrow. Liming was carried out three months before planting, by distributing the powder dolomitic limestone (90% total neutralizing power) all over the area with; the material was incorporated at 0.30 m depth at a dose corresponding to 2.4 x 10³ kg ha⁻¹, for both soils.

The soybean population of the cultivar MSOY-8001, during the spring of 2008; the seeds were manually spread over to get a stand of 444,444 plants ha⁻¹, with spacing of 0.45 m between rows and 20 seeds per meter along rows. The phosphorus fertilization was done at the planting time at a dose 120 kg ha⁻¹ P₂O₅ as superphosphate (18 mass% P₂O₅ and 10 mass% S) in all treatments and cultivation places. The seeds were inoculated with concentrated liquid inoculant (125 mL of liquid inoculant to 50 kg of seeds) o *Bradyrhizobium japonicum*. All cultural practices were followed as recommended by Embrapa (2008).

**Treatments and experimental design**

The crude glycerin was obtained from the conventional chemical industrial process of transesterification of triacylglycerides, in this case, of the cooking soybean oil after being used in frying foods. The transesterification was catalyzed with potassium hydroxide and the glycerin effluent was finally neutralized with sulfuric acid. The resulting liquid material will hereinafter be referred to as "K-glycerin". The chemical composition for this "K-glycerin" (pH = 8.3 and density = 1.01 kg dm³⁻¹), as could be determined according to analytical procedure described by Melo and Silva (2008), in kg m⁻³, is; P₂O₅ = 0.5; K₂O = 24.9; S = 1.1; B = 1 x 10⁻³; Cu = 4 x 10⁻⁵; Fe = 54 x 10⁻³; Mn = 2 x 10⁻³; Zn = 96 x 10⁻³ and organic carbonic = 3 x 10⁻³.

The field experimental design was a randomized block design with four replications. Each experiment plot consisted of four 3.0 m-long rows, spaced of 0.45 m, comprising a total area of 5.4 m². The useful area of each plot was considered to be the central two rows, excluding 1.0 m from each row-end.

The treatments consisted of four K₂O doses on the TH (0; 40; 80 and 160 kg ha⁻¹ K₂O) and TQ (0; 60; 120 and 240 kg ha⁻¹ K₂O) sites. These K₂O doses correspond to 0; 1.6; 3.2 and 6.4 m² ha⁻¹ "K-glycerin" on TH, and 0; 2.4; 4.8 "K-glycerin" and 9.6 m² ha⁻¹ on TQ. Two additional treatments were made to supply inorganic sources of potassium (KCl, corresponding to 58 mass% K₂O, and K₂SO₄, 48 mass% K₂O) at a dose of 80 and 120 kg ha⁻¹ K₂O on TH and TQ, respectively. These doses of potassium were based on recommendations for the soybean crop (Novais, 1999) and on the availability of potassium in the soil, evaluated according to standard procedures recommended for the soil chemical analysis (Table 1). The liquid "K-glycerin" was manually distributed all along the sowing rows at a depth of 7 cm.

**Inputs and measurements**

To evaluate the soybean crop yield, the grain moisture was corrected to 13 mass%. After harvesting the soybean grains, the soil samples were taken at a depth between 0 to 0.20 m from the soil surface, for each plot, in order to determine all other chemical properties. About 20 soil samples were collected from each plot in order to prepare one mixed sample. There were determined the pH in water, P and K (Mehlich-1 extractor), Ca and Mg (KCl 1 mol L⁻¹ extractor), H + Al (calcium acetate 0.5 mol L⁻¹ extractor) and the organic carbon (OC) according to the Walkley-Black method (Embrapa, 1997); the sulfate (SO₄²⁻) content was determined according to the procedure described by Hoefl et al. (1973). Values of cation exchange capacity (CEC = Σ(K, Ca, Mg, H + Al) in mmolc kg⁻¹) and saturation in bases (V% = [Σ(Ca, Mg, K)/(CEC)] x 100) were then calculated.

The nutritional status of the soybean leaf samples was determined at the stage R2 (full flowering) per useful plot, in order to determine the nutrient composition. The amount of nitrogen was determined by the semi-micro Kjeldahl method (Cunniff, 1995). The P, K, Ca, Mg, S, Cu, Fe, Mn and Zn contents were obtained by nitric perchloric acid digestion (Miller, 1998). The contents of Ca, Mg, Cu, Fe, Mn, and Zn were determined by atomic absorption spectrometry; K was determined by flame photometry (Isaac and Kerber, 1971). S was determined by the barium sulfate turbidimetry (Beaton et al., 1968). The B content was determined by colorimetry (azomethine method) after dry digestion (incineration) (Wolf, 1974).

Ten soil sub-samples were randomly collected at a depth between 0 and 0.10 m deep. The first emergence soybean plants were collected, packaged in plastic bags and transported in thermally isolated boxes. The materials were sieved (2 mm) in order to remove organic residues and roots. Then sub-samples were stored at 4°C until microbial analyses were made. Flurescein diacetate hydrolysis (FDA) by soil microorganisms was evaluated according to Frighetto and Valarini (2000). Soil basal respiration (Rbasal) was estimated based on CO₂ released from four sub-samples (20 g each) taken from each mixed soil sample (water content, 60 mass%). The samples were sealed in a 1.0 L flask with 10 mL of 0.3 mol L⁻¹ KOH and titrated, after three days, with 0.1 mol L⁻¹ HCl, according to the methodology described by Alef and Nannipieri (1995). Soil microbial biomass C (Cmic) was determined by fumigation and incubation, as described by Jenkinson and Powlson (1976). The metabolic quotient (qCO₂) was determined by the ratio between Rbasal and Cmic (Anderson and Domsch, 1993), expressed in μg CO₂ μg Cmic⁻¹ day⁻¹.

**Statistical analysis**

The numerical data were subjected to analysis of variance for the following factors: blocks, soil type, K₂O doses as "K-glycerin" and inorganic sources of potassium (KCl and K₂SO₄), as an additional nutrient supply. The microbiological analysis of soil, and the factors above, added two evaluation periods (early emergence and harvest) of the soybean. The separation of the mean values was done using the criterion of the least significant difference (LSD) at a 5% probability level. The fitted equations for the variables evaluated in terms of K₂O doses applied as "K-glycerin".

| Solo | pH_Water | P | K | S-SO₄²⁻ | Ca | Mg | Al | CEC | m | V | OC | Area | Silte | Argila |
|------|----------|---|---|--------|----|----|---|-----|---|---|----|------|-------|-------|-------|
| TH   | 5.8      | 1.4| 70| 4.8    | 23| 11| 7  | 83  | 16| 43| 17.4| 90   | 450   | 460   |
| TQ   | 5.6      | 1.8| 16| 7.1    | 5 | 2 | 11 | 37  | 60| 20| 4.0 | 890  | 60    | 50    |

pH_Water: Soil-water 1:2.5. P e K: Mehlich-1 extractor; Ca, Mg e Al: KCl 1 mol L⁻¹ extractor; S-SO₄²⁻: Calcium phosphate diacid (Ca(H₂PO₄)₂) extractor; CEC: Cation exchange capacity; m: Aluminum saturation; V: Bases saturation; OC: Organic carbon by the Walkley-Black method; Sand, silt and clay: Pipette method; TH: Typic Hapludox e TQ: Typic Quartzipsamment.
RESULTS AND DISCUSSION

Grain yield of soybean influenced by the K$_2$O doses in the form of "K glycerin" to increase quadratically when cultivated in soil TH and linearly when cultivated in soil TQ (Figure 1). The inorganic sources (KCl and K$_2$SO$_4$) applied in TH did not differ and when applied in TQ were superior and differed doses of K$_2$O in the form of "K glycerin" (Figure 1). The K$_2$O doses in "K glycerin" in applied TH caused reductions in soybean yield due to nutritional imbalance caused by competition cationic and anionic nutrients (Parker and Norvell, 1999). The lack of increase in grain yield of inorganic sources relative "K glycerin" and the latter by reduction with increasing K$_2$O doses in TH due to the initial K content in soil (Table 1), with a value above 40 mg kg$^{-1}$ of soil (Rosolem et al., 1993; Scherer, 1998a; Borket and Yamada, 2000).

Differently than soybean cultivation in TH, response to K fertilization with application of "K glycerin" and inorganic sources occurred when exchangeable soil K was below 40 mg kg$^{-1}$ in TQ soil. Responses to K fertilization in soybean were found in Typical Hapludox with K content of 27.3 mg kg$^{-1}$ of soil (Mascarenhas et al., 2000).

The maximum yield (1,157 kg ha$^{-1}$) of soybean attained with the maximum dose applied K$_2$O (240 kg ha$^{-1}$) in the form of "K glycerin" on the ground TQ (Figure 1). In soil TH, maximum yield of 1,622 kg ha$^{-1}$ attained with 87 kg ha$^{-1}$ of K$_2$O with "K glycerin" (Figure 1). The K$_2$O dose were higher than the determined by Scherer (1998b) and the near recommended for soybean (Novais, 1999) and obtained in succession millet-soybean no-tillage (Foloni and Rosolem, 2008) to achieve maximum productivity in TH soil. In TQ soil, the K$_2$O dose as "K glycerin" to achieve maximum yield was more elevated than in TH soil.

The yield of soybean crop in Brazil in 2012/2013 was 2,938 kg ha$^{-1}$ and the Minas Gerais State, Brazil was 3,010 kg ha$^{-1}$ (Conab, 2013) with fertilizer application in soybean seeding. The conditions of this study, the yields on both cultivated soils were significantly lower than the national and regional average. The results attributed to climatic conditions during the experimental period with a mean temperature of 24.2 and 20.0$^\circ$C and rainfall of 735 and 710 mm in Curvelo and Diamantina, respectively. The favorable climatic factors are essential for proper yield of soybean with water requirement of 7 to 8 mm per day, totaling in cycle from 450 to 800 mm, depending on the duration and management of the crop cycle and optimal temperature around 30$^\circ$C (Embrapa, 2008). The temperature interfered on soybean yield in both cultivation places, with greater effect in Diamantina, besides the difficulty of K fertilizer management due to the low CEC in TQ soil (Table 1), which provides low yield with higher K dose applied to the soil. The need for application of dose above 60 kg ha$^{-1}$ of K$_2$O in soils with low CEC and in areas subject to intense rainfall, K should be applied broadcast at doses allowing maintenance of adequate levels in soil and that return the quantities exported by crops (Guareschi et al., 2008).

The direct application of "K glycerin" was evaluated as an alternative source of nutrients for the soybean crop, thus the soil chemical attributes and levels in the leaves

Figure 1. Grain yield of soybeans as a function of K$_2$O doses in the form of "K glycerin" (KG) and K inorganic sources (KCl and K$_2$SO$_4$) into two types of soil (*Significant at 1% by F-test).
of soybean was evaluated (Tables 2 and 3). The K and S-SO₄²⁻ contents in soil affected quadratically (K: ŷ = 75.2 + 1.19x - 0.006x², R² = 0.95 and S-SO₄²⁻: ŷ = 13.4 + 0.35x - 0.002x², R² = 0.98) in TH and linearly in TQ (K: ŷ = 19.0 + 0.15x, R² = 0.98 and S-SO₄²⁻: ŷ = 19.9 + 0.05x, R² = 0.99) by increasing K₂O doses of “K glycerin” that differed from K inorganic sources (KCl and K₂SO₄) applied (Table 2). In contrast, the K in soybean leaves only was affected linearly (K: ŷ = 7.2 + 0.0171x, R² = 0.92) by increasing K₂O doses of “K glycerin” in TQ which differed from K inorganic sources applied (Table 3). Foliar S increased with K₂O doses of “K glycerin” linearly in TH soil (S: ŷ = 0.8 + 0.0046x, R² = 0.95) and in TQ soil (S: ŷ = 1.2 + 0.0016x, R² = 0.89) and differed from K inorganic sources in both soils (Table 3).

The diagnosis of a nutrient deficiency, it is important to conduct soil testing in advance, to make possible corrections in the fertilization to minimize future losses in yield. Soybean yield (Figure 1) had a relationship with the available K and S content in the soils (Table 2). The exchangeable soil K in TH by initial analysis (Table 1) is above 40 mg K kg⁻¹ soil whereas the TQ is below (Rosolem et al., 1993; Scherer, 1998a; Borket and Yamada, 2000). In contrast, the availability of the S-SO₄²⁻ in the initial analysis (Table 1) is above 9.3 mg S-SO₄²⁻ kg⁻¹ soil (Huda et al., 2004) in both soil cultivated with soybeans. This S and K availability in the soil reflected in the content of such nutrients in the leaves of soybean (Table 3). The contents of K and S in the soil increased with “K glycerin” doses since these nutrients constituents of this waste arising from the production process of biodiesel used, with the K inorganic sources and due to P fertilization of soybean with superphosphate.

The increases in S and K in the soil (Table 2) as reflected in contents of these nutrients in the leaves of soybean (Table 3) provided no increases in grain yield in TH (Figure 1), due to S content in the leaves of soybean being below the 2.5 g kg⁻¹ amount (Martinez et al., 1999) and 2.3 g kg⁻¹ (Uran et al., 2007). The nutrient with the greatest increase in TQ both in soil (Table 2) as in the leaves of soybean (Table 3) was the K thereby could be responsible for increased yield. The K influences various physiological processes such as photosynthesis, transport of photosynthesates and enzymes activation, which directly affected the yield (Pettigrew, 2008). The K content in the leaves below 14.0 g kg⁻¹ (Scherer, 1998a), of 17.0 g kg⁻¹ (Martinez et al., 1999) and 23.1 g kg⁻¹ (Uran et al., 2007) related to low yield of soybeans (Figure 1) at the maximum dose of “K glycerin” in TQ.

The effect of K in Ca and Mg uptake, which normally interact with this nutrient (Mascarenhas et al., 1988), decreased the levels of Ca and Mg in the leaves of soybean when cultivated in TQ (Table 3). The difference of Ca and Mg in TQ may be due to competition with K, since they use the same absorption sites (Andreotti et al., 2001), and the increase of K intensifies competition with
Ca and Mg (Oliveira et al., 2001). In TH, the high content of K in the initial analysis (Table 1) probably equated to the Ca and Mg competition occurring balanced as opposed to TQ, with higher competition between the exchangeable bases.

Despite the increase in K and S in soil (Table 2) and in leaves of soybean (Table 3), application of the "K glycerin" was not sufficient to modify the CEC and the organic carbonic content in two soils (Table 2). A single application of the "K glycerin" in planting furrow added at a dose of 240 kg ha\(^{-1}\) K\(_2\)O in TQ a quantity of 28.9 kg ha\(^{-1}\) of organic carbonic, being required to maintain the initial stock carbon organic soil a total annual addition of 8,900 kg ha\(^{-1}\) carbon in tillage cropping system (Lovato et al., 2004). The increase in organic matter and consequent increase of soil CEC by addition of organic waste was obtained when applied to the total area (Carvalho et al., 2011) and the use of organic waste (poultry manure) did not affect the organic matter in depth up to 0.20 m (Moreti et al., 2007), while the organic matter effect restricted to the surface layers of the soil, which not reflected in the 0 to 0.20 m soil due to the dilution effect on the soil mass (Ciotta et al., 2003; Araújo et al., 2007).

Microbiological analyzes were performed at two different times in order to obtain information regarding the direct application of "K glycerin" in soil microbes (Table 4). The reduction of microbial activity measured by fluorescein diacetate hydrolysis (FDA) with increasing K\(_2\)O doses (FDA: ŷ = 4.36 - 0.019x + 0.00012x\(^2\), R\(^2\) = 0.83) in the form of "K glycerin" in TH (Table 4) and the source K\(_2\)SO\(_4\) may have occurred because the dose was detrimental to soil microbes at the time of soybean emergence (E1). The addition of organic and inorganic fertilizers can cause positive or negative effects on microbial biomass and its activity (Böhme et al., 2005).

It was observed reduction value of the FDA at the time of harvest of soybean (E2) in relation to the time E1 did not differ between the application of "K glycerin" doses and K inorganic sources in TH (Table 4). Already in the soil TQ, microbial activity was not influenced by the levels of "K glycerin" and K inorganic sources at the time E1 and an increase in the time that E2 (FDA: ŷ = 1.98 + 0.002x, R\(^2\) = 0.82) did not differ from K inorganic sources (Table 4). The evaluation times, the behavior was different in relation to TH because TQ on the end of the experimental period the value was different at p<0.05.

### Table 3. Effect of K\(_2\)O doses in the form of "K Glycerin" (KG) and inorganic sources (KCl and K\(_2\)SO\(_4\)) on nutrient concentrations in the soybean leaves applied to two types of soil.

| Treatment | N  | P  | K  | Ca | Mg | S   | B  | Cu | Fe | Mn | Zn |
|-----------|----|----|----|----|----|-----|----|----|----|----|----|
|           | g kg\(^{-1}\) | mg kg\(^{-1}\) |
| Typic Hapludox: KG doses (kg K\(_2\)O ha\(^{-1}\)) | | | | | | | | | | | |
| 0         | 36\(^a\) | 2.4\(^a\) | 21\(^a\) | 10\(^a\) | 2.2\(^a\) | 0.9\(^a\) | 33\(^a\) | 9\(^a\) | 78\(^a\) | 23\(^a\) | 30\(^a\) |
| 40        | 36\(^a\) | 2.4\(^a\) | 20\(^a\) | 10\(^a\) | 2.2\(^a\) | 0.8\(^a\) | 32\(^a\) | 8\(^a\) | 74\(^a\) | 25\(^a\) | 28\(^a\) |
| 80        | 38\(^a\) | 2.5\(^a\) | 21\(^a\) | 10\(^a\) | 2.2\(^a\) | 1.3\(^a\) | 31\(^a\) | 10\(^a\) | 69\(^a\) | 21\(^a\) | 31\(^a\) |
| 160       | 38\(^a\) | 2.5\(^a\) | 20\(^a\) | 11\(^a\) | 2.2\(^a\) | 1.5\(^a\) | 32\(^a\) | 9\(^a\) | 66\(^a\) | 24\(^a\) | 28\(^a\) |
| KCl (80 kg K\(_2\)O ha\(^{-1}\)) | 39\(^a\) | 2.5\(^a\) | 21\(^a\) | 10\(^a\) | 2.2\(^a\) | 0.9\(^a\) | 31\(^a\) | 9\(^a\) | 68\(^a\) | 22\(^a\) | 30\(^a\) |
| K\(_2\)SO\(_4\) (80 kg K\(_2\)O ha\(^{-1}\)) | 36\(^a\) | 2.5\(^a\) | 21\(^a\) | 11\(^a\) | 2.2\(^a\) | 1.6\(^a\) | 31\(^a\) | 12\(^a\) | 76\(^a\) | 23\(^a\) | 30\(^a\) |
| F-test    | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

| Typic Quartzipsamment: KG doses (kg K\(_2\)O ha\(^{-1}\)) | | | | | | | | | | | |
| 0         | 43\(^a\) | 3.2\(^a\) | 7\(^b\) | 17\(^a\) | 4.6\(^a\) | 1.1\(^b\) | 22\(^a\) | 12\(^a\) | 63\(^a\) | 37\(^a\) | 28\(^a\) |
| 60        | 41\(^a\) | 3.1\(^a\) | 8\(^b\) | 17\(^a\) | 4.6\(^a\) | 1.4\(^b\) | 22\(^a\) | 11\(^a\) | 63\(^a\) | 37\(^a\) | 28\(^a\) |
| 120       | 45\(^a\) | 3.1\(^a\) | 10\(^b\) | 17\(^a\) | 4.2\(^a\) | 1.5\(^b\) | 21\(^a\) | 13\(^a\) | 69\(^a\) | 36\(^a\) | 28\(^a\) |
| 240       | 44\(^a\) | 3.1\(^a\) | 11\(^b\) | 15\(^a\) | 4.2\(^a\) | 1.5\(^b\) | 21\(^a\) | 12\(^a\) | 70\(^a\) | 36\(^a\) | 28\(^a\) |
| KCl (120 kg K\(_2\)O ha\(^{-1}\)) | 45\(^a\) | 3.1\(^a\) | 20\(^b\) | 11\(^a\) | 2.6\(^a\) | 1.6\(^b\) | 22\(^a\) | 12\(^a\) | 78\(^a\) | 39\(^a\) | 27\(^a\) |
| K\(_2\)SO\(_4\) (120 kg K\(_2\)O ha\(^{-1}\)) | 42\(^a\) | 3.0\(^a\) | 20\(^b\) | 12\(^a\) | 2.6\(^a\) | 1.9\(^b\) | 22\(^a\) | 12\(^a\) | 79\(^a\) | 39\(^a\) | 27\(^a\) |
| F-test    | NS | NS | * | NS | NS | * | NS | NS | NS | NS | NS |
| CV (%)    | 7.9 | 13.4 | 10.4 | 13.2 | 3.7 | 11.3 | 12.2 | 5.4 | 19.4 | 17.4 | 19.2 |

NS: Non-significant at p<0.05; *: significant at p>0.05. Means with the same treatment and column sharing the same letters are not significantly different at p<0.05.
Table 4. Effect of K2O doses in the form of “K Glycerin” (KG) and inorganic sources (KCl and K2SO4) on microbiological analyzes in the emergency (E) and harvest (H) of soybean applied to two types of soil.

| Treatment | FDA (mg kg⁻¹ h⁻¹) | Rbasal (mg kg⁻¹ h⁻¹ CO2) | Cmic (mg kg⁻¹ soil) | qCO₂ (µg CO₂ µg Cmic⁻¹ day⁻¹) |
|-----------|---------------------|--------------------------|---------------------|---------------------------------|
|           | Emergency    | Harvest       | Emergency    | Harvest       | Emergency    | Harvest       | Emergency    | Harvest       |
| Typic Hapludox: KG doses (kg K2O ha⁻¹) | | | | | | | | |
| 0         | 4.27±       | 0.69±        | 1.29±       | 0.69±        | 374.4±     | 389.6±       | 0.08±       | 0.04±       |
| 40        | 4.00±       | 0.91±        | 1.54±       | 0.90±        | 112.8±     | 241.5±       | 0.33±       | 0.09±       |
| 80        | 3.39±       | 1.14±        | 3.09±       | 0.95±        | 149.7±     | 155.5±       | 0.49±       | 0.14±       |
| 160       | 4.33±       | 1.07±        | 3.46±       | 1.00±        | 187.1±     | 137.2±       | 0.44±       | 0.17±       |
| KCl (80 kg K2O ha⁻¹) | 4.59±       | 1.06±        | 2.41±       | 0.96±        | 214.5±     | 119.8±       | 0.27±       | 0.19±       |
| K2SO4 (80 kg K2O ha⁻¹) | 3.34±       | 1.05±        | 2.39±       | 1.09±        | 273.5±     | 125.3±       | 0.21±       | 0.21±       |
| F-test    | *           | NS           | *           | NS           | *           | NS           | *           | NS           |
| CV (%)    | 15.1        | 17.1         | 9.2         | 35.6         |

NS: Non-significant at p<0.05; *: Significant at p>0.05. Means with the same treatment and column sharing the same letters are not significantly different at p<0.05. FDA: Flurescein diacetate hydrolysis; Rbasal: Soil basal respiration; Cmic: Soil microbial biomass carbonic; qCO₂: Metabolic quotient.

0.94) in TQ, which only differed between the two K inorganic sources in the last soil (Table 4). The application of organic waste (“K glycerin”) Rbasal increased due to addition of organic C and nutrient (Lambais and Carmo, 2008) in both soils. The K inorganic sources (KCl and K2SO4) did not stimulate the soil microbes and the “K glycerin” doses were not sufficient to promote higher activity regarding the K mineral sources in the TH soil because the increased soil microbial activity depend on carbon available soil (Araújo and Monteiro, 2006). In soybean harvest (E2), TQ soil only increased the Rbasal with increasing doses of “K glycerin” that differed from K inorganic sources (Table 4). The moisture stress occurred during the experimental period in the cultivation of soybean decreased microbial activity in TH. The higher doses of “K glycerin” apply the TQ may not have been completely decomposed, with a residual effect of C organic in soil. The organic matter added to the soil in the form of organic waste according to the degree of decomposition, may have an immediate or residual soil by means of a slower process of decomposition (Santos et al., 2001).

In soybean emergence (E1), the soil microbial biomass carbonic (Cmic) in TH were reduced quadratic “K glycerin” doses (Cmic: ŷ = 348.94 - 5.30x + 0.027x², R² = 0.90) that differed from K inorganic sources (Table 4), may have been due to an effect of “K glycerin” that harmed the soil microbes. At harvest of soybean (E2) in TH, due to lack of rainfall during the experimental period, the Cmic values were lower at the time E1, with a quadratic decrease with increasing doses of “K glycerin” (Cmic: ŷ = 388.71 - 4.30x + 0.017x², R² = 0.99), which differ from the inorganic sources of K (Table 4). In TQ, the behavior of the Cmic was different, showing that “K glycerin” stimulated the growth of microbes quadratically in both evaluation periods E1 (Cmic: ŷ = 145.86 + 2.78x - 0.012x², R² = 0.99) and E2 (Cmic: ŷ = 216.42 + 0.83x - 0.003x², R² = 0.95), but the K inorganic sources (KCl and K2SO4) caused some effect detrimental microbial growth, with reduced Cmic (Table 4). Environmental factors among them such as soil moisture, can modify the ecology, population dynamics and soil microbial activity due to modification of the microbial habitat (Nannipieri et al., 2003).

The metabolic quotient (qCO₂) valuated in soybean emergence (E1) may verify that the application of “K glycerin” doses and K inorganic sources caused more stress in both soils (Table 4). Already at the time of harvest of soybean (E2), the evaluation can verify the stabilizing trend in both soils due to exhaustion of nutrients, thus achieving a balance in the middle, by reducing stress soil (Moreno et al., 1999). The application “K glycerin” caused no major changes in the soil chemistry unless the concentration of K and S is a component of this organic waste arising from the production process of biodiesel. Microbiological analysis
of soil showed a higher when stress was applied and reducing the end of the experimental period. The values of qCO₂ indicate that the use of “K glycerin” does not cause stress in the soil, thus can be used without major environmental consequences related to their direct application to the soil.

The use of “K glycerin” in the field of soybean yield in soils with average K content, resulting in the recommendation of 87 kg ha⁻¹ of K₂O to achieve maximum grain yield would result in the removal of 3.5 m² ha⁻¹ crude glycerin in the biodiesel industry stocks. The area planted with soybeans in Brazil in the harvest of 2012/2013 was just over 28 million ha (Conab, 2013); thus 1% of this area was used to “K glycerin” would remove national stocks a total of 98 million m³ of glycerin, making this waste not disposed to harming the environment. The “K glycerin” does not cause environmental problems to the place where it was applied as recommended by the available K in soil, however, studies are needed to evaluate its long-term use to understand the dynamics of the crop yield and soil.

Conflict of Interest
The authors have not declared any conflict of interests.

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