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# ACTIVITY OF RHIZOSPHERE SOIL MICROORGANISMS OF SUGARCANE CULTIVARS AFTER SPRAYING OF HERBICIDES: DIURON, TEBUTHIURON, AMETRYN AND DIURON + HEXAZINONE[1]

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**ABSTRACT** - Changes in the agricultural environment can be determined by providing microbiological indicators of the soil since the soil microorganisms are sensitive to variations in the environment. In this way, the impact of herbicides of long residual effect on the rhizospheric soil microorganisms of sugarcane cultivars was evaluated. The cultivars of sugarcane (SP 81-3250 and RB 867515) were treated with four herbicides (tebuthiuron, diuron, ametryn and mixture of diuron + and hexazinone) applied in pre-emergence. The herbicides were applied seven days after the planting of the gems. At 30, 60 and 90 days after the application, the soil rhizosphere was collected in each treatment to determine the CO2 evolution of the soil (C-CO2), microbial biomass carbon (MBC), metabolic quotient (qCO2), solubility potential of inorganic phosphorus P (PSFI) and percentage of roots colonized by mycorrhizal fungi Arbuscular. No changes were observed in the microbial activity of the sugarcane rhizosphere at 30 days after application of the herbicides (DAA). However, at 90 DAA, all herbicides negatively affected the activity of the rhizospheric microorganisms of sugarcane. The metabolic activity of rhizosphere in soil cultivated with RB 867515 was less affected by herbicides.

**Keywords**: Rhizosphere. Soil microorganisms. Residual effect.

## ATIVIDADE DA MICROBIOTA RIZOSFÉRICA DE CULTIVARES CANA-DE-AÇÚCAR APÓS APLICAÇÃO DOS HERBICIDAS: DIURON, TEBUTHIURON, AMETRYN E DIURON + HEXAZINONE

**RESUMO** - Alterações no ambiente agrícola podem ser determinadas por meio indicadores microbiológicos do solo, uma vez que a microbiota do solo é sensível às modificações do ambiente. Desta forma, avaliou-se o impacto de herbicidas de longo efeito residual sobre a microbiota rizosférica de cultivares de cana-de-açúcar. As cultivares de cana-de-açúcar SP 81-3250 e RB 867515 foram tratadas com quatro herbicidas (tebuthiuron, diuron, ametryn e a mistura diuron + hexazinone) aplicados em pré-emergência. Os herbicidas foram aplicados sete dias após o plantio das gemas. Aos 30, 60 e 90 dias após a aplicação, o solo rizosferico de cada tratamento foi coletado para determinar a evolução de $CO_2$ no solo ($C\text{-}CO_2$), o carbono da biomassa microbiana (MBC), o quociente metabólico ($qCO_2$), o potencial de solubilização de P inorgânico (PSFI) e a percentagem de raízes colonizadas por fungos micorrízicos arbusculares. Não foram observadas alterações na atividade microbiana da rizosfera da cana-de-açúcar aos 30 dias após a aplicação dos herbicidas (DAA). No entanto, aos 90 DAA todos os herbicidas afetaram negativamente a atividade dos micro-organismos rizosféricos da cana-de-açúcar. A atividade metabólica da rizosfera em solo cultivado com a RB 867515 foi menos afetada pelos herbicidas.

**Palavras-chave**: Rizosfera. Microrganismos do solo. Efeito residual.

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## INTRODUCTION

The herbicides are widely used in different crops, but, abusive use and lack of knowledge of the interactions between these products with the environment after its application may cause disturbances that alter the environmental balance (DAS; DEBNATH; MUKHERJEE, 2003; RIZZARDI et al., 2003). Many of these effects in the environment are not perceptible, therefore, before an herbicide application, it is necessary detailed evaluations of risks for safe recommendations (RIZZARDI et al., 2003).

The herbicides when applied inevitably reach the soil (NETO et al., 2017) promoting changes in the microbial community (REIS et al., 2008; RÉGO et al., 2014). The imbalance in the microbial community may result in adverse effects on the development of the crop since these organisms are capable of improving the chemical, physical and biological properties of the soil (POEPLAU; DON, 2015). Some microorganisms can promote nitrogen fixation and solubilization of inorganic phosphate in the soil, providing nutrients for crop growth and development (FREITAS; BANERJEE; GERMIDA, 1997). Other microorganisms are involved in the biogeochemical cycle of elements such as K, Ca, Mg and S that are essential for cultivated plants (TÓTOLA; CHAER, 2002; GIL-STORES et al., 2005).

The application of insecticides and herbicides promoted an imbalance in the soil microorganisms, altering the mineral cycling, as well as C/N ratio of soil (ISLAM; WEIL, 1998; REIS et al., 2008; GRAYSTON; VAUGHAN; JONES, 1996; NAUTIYAL, 1999). The composition of the microbial community varies with species or crop cultivated in the area (REIS et al., 2008). Some cultivated species may provide optimal conditions for growth of specific microorganisms in the soil, and these often accelerate the degradation of herbicides (VILLAVERDE et al., 2017). Moreover, this interaction between cultivar and soil microorganisms can mitigate the adverse effects caused by the herbicide applications to the soil microorganisms itself (VILLAVERDE et al., 2017). Thus, the evaluation of the microbiological activity of the soil can be used to evaluate the environmental impacts generated by herbicides in different agroecosystems (TÓTOLA; CHAER, 2002; GIL-STORES et al., 2005).

The safe recommendation of herbicides should consider the efficiency to control weeds, as well as the interactions of these pesticides with the environment, so that these compounds, when applied, generate the least possible environmental impact. The objective of this work was to evaluate the impact of the herbicides diuron, tebuthioron, ametryn and diuron + hexazinone in the soil microbial community and the mycorrhization of two sugarcane cultivars.

## MATERIAL AND METHODS

The experiment was carried out in a greenhouse, with each experimental unit corresponding to a plastic vessel with a capacity of 15 $dm^{-3}$ filled with samples of a Red-Yellow Argisol (Table 1). The soil was collected in an area without herbicide application history.

The experimental design was completely randomized, with four replications. The treatments were arranged in a 3 x 5 x 2 factorial scheme, with the first factor corresponding to the sampling times (30, 60 and 90 days after application). The second factor was applied herbicides (ametryn, diuron, tebuthiuron and diuron + hexazinone ), also, a control without herbicide application, and the third to sugarcane cultivars (RB-85715 and SP 81-3250).

The applied dose of each herbicide varied as recommended by the package leaflet: ametryn (2.5 g ia $ha^{-1}$), diuron (2.4 g ia $ha^{-1}$) ), tebuthiuron (2.0 g ia $ha^{-1}$), diuron + hexazinone (1.170 + 330 g ia $ha^{-1}$).

**Table 1**. Chemical and physical analysis of soil.

| Solo | | Areia | | Silte | | Argila | | Classe Textural | | | P-rem | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ----------dag $kg^{-1}$---------- | | | | | | | | |
| PVA | | 20 | | 27 | | 53 | | Argissolo | | | 21.3 | |
| pH | P | K | Ca | Mg | Al | H+Al | SB | (t) | T | V | m | MO |
| $H_2O$ | --mg $dm^3$-- | | | | -------$cmol_c$ $dm^3$------- | | | | | | ---%--- | dag $kg^{-1}$ |
| 5.2 | 14.3 | 162 | 3.6 | 0.7 | 0.1 | 3.14 | 4.71 | 4.81 | 7.85 | 60 | 2 | 3.6 |

Analysis carried out in the Laboratory of Analysis of Soil Viçosa, according to the methodology of the Brazilian Agricultural Research Company - EMBRAPA (1997); PVA = Yellow Red Argisol; SB = sum of bases (t) = effective cation exchange capacity; T; V = base saturation; m = Saturation by Al + 3; MO = organic matter.

Samples of rhizosphere soils were collected at 30, 60 and 90 days after application of herbicides (DAA). After each collection, they were taken to the Laboratory of Herbicides in Soil and incubated to evaluate the $CO_2$ emission and microbial biomass.

In the soil respiration rate, the respirometric method was used to evaluate the evolved C-$CO_2$ of the soil. For this, samples of 100 g of moist soil (70% of field capacity) were sieved and incubated for 15 days in closed flasks. The C-CO2 released from the soil was carried by a continuous flow of air ($CO_2$-free) to another flask containing 100 mL of 0.5 mol $L^{-1}$ NaOH solution. At the end of incubation (15th day), the evolved C-$CO_2$ was estimated indirectly by titration of 10 mL of NaOH solution, used to capture $CO_2$, with a 0.5 mol $L^{-1}$ HCl solution. A control to monitor the air quality was added in respirometry system, using bottles without soil.

After 15 days of incubation, the soil was removed from the flasks, taking 18 g of soil from each flask to determine the microbial biomass carbon (MBC). The method used was described by Vance et al. (1987), modified by Islam and Weil (1998), in which half samples were treated with microwave radiation for 120 seconds (60 + 60 s). The MBC was extracted from the soil samples (irradiated and non-irradiated) with 80 mL of the $K_2SO_4$ solution (0.5 mol $L^{-1}$) under agitation for 30 min in a horizontal table and then held for 30 min.

After this process, the supernatant was filtered on Whatman Nº 42 paper. 10 mL of the filtrate was added and flask with 2 mL of $K_2Cr_2O_7$ (0.0667 mol $L^{-1}$) and 10 mL of $H_2SO_4$ P.A. solution to promote the sulfuric digestion of the soil organic carbon. Subsequently, the volume was filled to 100 mL with distilled water. The samples were transferred to 250 mL Erlenmeyer, adding the Ferroin indicator (eight drops), titrating with $(NH_4)_2Fe(SO_4)_2$ (0.033 mol $L^{-1}$) until the solution showed a brick red color. The metabolic quotient ($qCO_2$) was calculated by dividing the daily mean of the evolved C-$CO_2$ of the soil by the MBC determined in the soil.

To determine mycorrhizal colonization, the root system of sugarcane plants was collected at 30, 60, and 90 days after herbicides application. Samples of the root system were collected and stored in FAA solution 1:1:18 (formaldehyde: acetic acid: ethyl alcohol). Subsequently, the roots were placed in a flask with 10% KOH, lactoglycerol, and trypan blue, at 70 °C, to color the roots and fungal structures. The root segments were arranged on a petri dish with a checkered background in which they were evaluated and classified as colonized or non-colonized by mycorrhizal fungi. In the end, the percentage of mycorrhizal roots was determined.

The solubilizing inorganic phosphate potential was evaluated, using 1 g of soil obtained of the rhizospheric of each treatment. The soil samples were added in tubes with NBRI liquid medium, at pH 6.8 to 7.0, containing (g $L^{-1}$): 10 g $L^{-1}$ of glucose; 5 g $L^{-1}$ of $Ca_3(PO_4)_2$; 0.5 g $L^{-1}$ of $MgCl_2.6H_2O$; 0.25 g $L^{-1}$ of $MgSO_4.7H_2O$; and 0.1 g $L^{-1}$ of $(NH_4)2SO_4$ (NAUTIYAL, 1999). These samples were incubated for 15 days at 27 °C, and after this period, they were centrifuged at 8,000 rpm for 20 min. The inorganic P was determined in the supernatant by modified vitamin C colorimetric method, at the wavelength of 725 nm (BRAGA; DE FELLIPO, 1974).

The means of microbiological data were submitted to analysis of variance and, when significant, the means were compared by the Tukey test at 5% of significance.

## RESULTS AND DISCUSSION

The microbiological variables of rhizospheric soil were influenced by the interaction between herbicides x cultivars x season. The diuron application increased the $CO_2$ emission in rhizosphere soils of the cultivar RB 86751, at 30 days after application (DAA) (Figure 1).

This fact indicates that in the presence of this herbicide, the microorganisms underwent a stress, increasing its respiration rate. However, the same herbicide in mixture with hexazinone did not alter the microorganisms respiration. The differential response of microorganisms when exposed to the herbicides diuron, and diuron + hexazinone may be caused by the different doses of diuron applied in each treatment. In the treatments where the mixture was applied, the dose of diuron was lower compared to the isolated application of this herbicide, and consequently, a lower $CO_2$ emission was also observed. Studies have shown that different microorganisms inoculated in different soils promoted the diuron degradation, liberating $CO_2$ for atmosphere (VILLAVERDE et al., 2017). This effect was also observed in soils not inoculated with specific microorganisms (VILLAVERDE et al., 2017). Thus, the most significant $CO_2$ emission in the treatments where the diuron isolated was applied may be due to the elevated amount of substrate available for mineralization of this herbicide by the soil microorganisms.

Tebuthiuron reduced the $CO_2$ emission and MBC in rhizosphere soil of the cultivar SP 81-3250 compared to cultivar RB85715 at 60 DAA (Figure 1 and Figure 2). This herbicide may reduce MBC due to the sensitivity of some microorganisms to this herbicide (PIRES et al., 2005). Thus, the lower evolution of $CO_2$ in the rhizosphere of SP 81-3250 is a result of the smaller population of microorganisms in these environments.

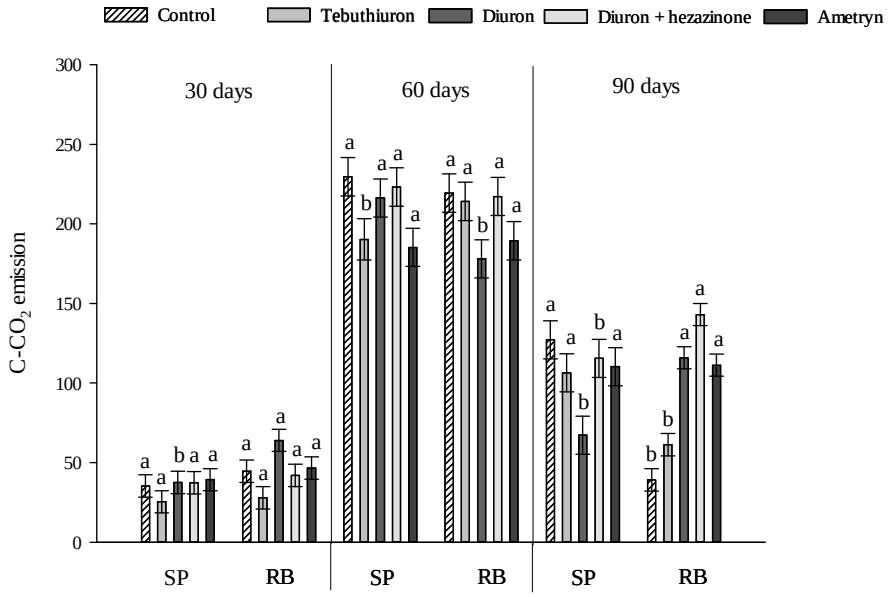


**Figure 1**. C-$CO_2$ emission of the rhizospheric soil of two sugarcane cultivars at 30, 60 and 90 days after herbicides application in pre-emergence. Lowercase letters differ the effect of each cultivar, and error bars differ the effect of each herbicide within the cultivar by the Tukey test at 5% probability.

The respiratory rate of the soil microorganisms present in rhizosphere soil of the SP 81-3250 cultivar was reduced by the tebuthiuron and ametryn application at 60 DAA. However, these herbicides did not affect the $CO_2$ emission at 60 DAA (Figure 1). Although the soils are cultivated with the same plant species (sugarcane), the microbial community that develops and grows in the soil can vary among different cultivars (PATHAN et al., 2015; SZOBOSZLAY et al., 2015). The microorganisms present in soils cultivated with cultivar SP 81-3250 presented a lower evolution of $CO_2$, without alteration in MBC, indicating a higher tolerance them to tebuthiuron and ametryn compared to the microorganisms that develop in cultivated soils with cultivar RB85715 at 60 DAA.

The reduction in the $CO_2$ emission, without changes in the MBC and $qCO_2$ values, in cultivated soil with SP 81-3250 indicates a lower equilibrium perturbation, showing that the application of these herbicides has little effect on the microbial community established in soils cultivated with this cultivar. This fact indicates that a smaller amount of $CO_2$ is lost to the atmosphere. Agronomic systems that emit lower amounts of $CO_2$ are considered conservative and contribute to reducing the harmful effects of increased $CO_2$ concentration in the atmosphere, such as the greenhouse effect (POEPLAU; DON, 2015).

Diuron + hexazinone and ametryn application increased the respiratory rate of the cultivar RB85715 and SP 81-3250 (Figure 1). Also, these herbicides reduced CBM compared to the control, without herbicide application, at 90 DAA (Figure 2). These herbicides can provocate an imbalance in the microbial community, leading to the death of some genera of microorganisms in the soil, thereby reducing CBM to 90 AAD. Studies evaluated the effect of these herbicides on different soils, founding a toxicity of these agrochemicals to some soil microorganisms (ABBATE et al., 2013; PROCÓPIO et al., 2014). However, the surviving population may have used the dead microbial population as a source of C and energy in their metabolism, resulting in increased of C-$CO_2$ production observed in this work (REIS et al., 2008).

Ametryn application did not alter the C-$CO_2$ emission and CBM in cultivated soils with cultivar SP 81-3250 at 60 DAA, however, an imbalance was evidenced in these plots at 90 DAA. Some herbicides, when applied, can cause immediate physiological changes in plants that, over time, can alter the rhizosphere and activity of the microorganisms (ANDERSON; KRUGER; COATS, 1994; MIMMO et al., 2014). However, the sugarcane cultivar used in this work are highly selective to ametryn. This herbicide did not alter the physiological and productive characteristics of SP 81-3250 (FARIA et al., 2013; BERTOLINO; ALVES 2014; FERREIRA et al., 2005), indicating that the highest $CO_2$ emission at 90 DAA is due to the direct effect of the herbicide on the soil microorganisms.

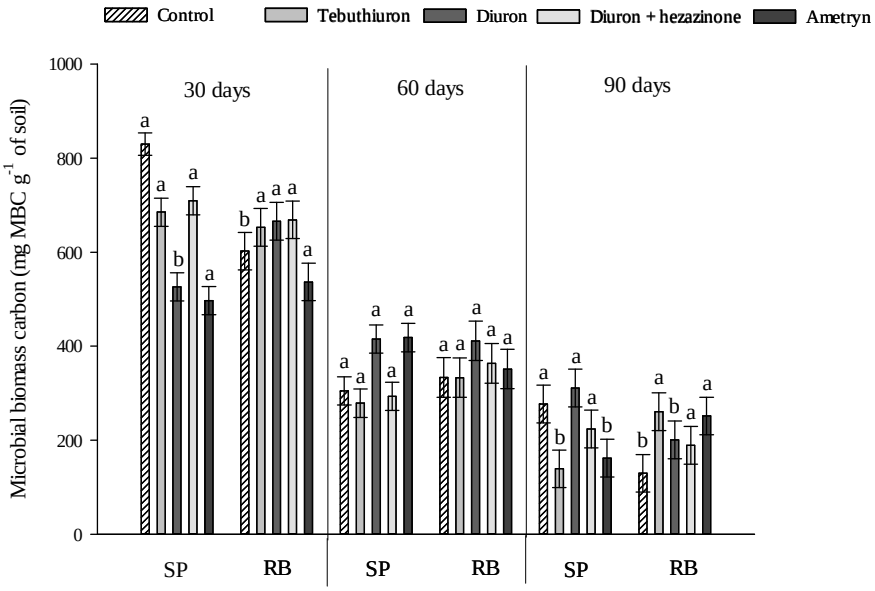


**Figure 2**. Microbial biomass carbon of rhizospheric soil of two sugarcane cultivars at 30, 60 and 90 days after herbicides application in pre-emergence. Lowercase letters differ the effect of each cultivar, and error bars differ the effect of each herbicide within the cultivar by the Tukey test at 5% probability.

The response time of the microbial community that develops in soils cultivated with SP 81-3250 was not immediate, and the greatest $CO_2$ emission in soils treated with ametryn may arise as the microorganisms are exposed to this agrochemical. A study evaluated the evolution of $CO_2$ in a soil cultivated with sugarcane submitted to the ametry application. In this work an exponential growth in $CO_2$ emission was observed only 100 days after the application of the herbicide, and this effect was not observed in the initial periods (RÉGO et al., 2014). In addition, these authors associated the highest $CO_2$ evolution with the degradation of the ametryn, since the degradation rate of this herbicide presented a behavior directly proportional to the $CO_2$ emitted from the soil (RÉGO et al., 2014). Thus, the higher $CO_2$ evolution found at 90 DAA may be a result of the increased in the ametryn degradation rate.

The herbicides ametryn, tebuthiuron, diuron and hexazinone are inhibitors of FS II and can affect the growth of microorganisms (REIS et al., 2008). These authors reported a toxicity of these herbicides on soil microbial biomass. These herbicides act to inhibit photosynthesis and cause deleterious effects on photosynthetic microorganisms. However, the effect of photosystem II inhibitor herbicides is questionable for soil microorganisms because most of them are not photoautotrophic, in other words, they cannot fix atmospheric $CO_2$.

All the treatments did not alter the soil metabolic quotient at 30 DAA (Figure 3). The herbicide diuron reduced the metabolic quotient of the rhizosphere of both sugarcane cultivars, whereas ametryn reduced the value of this variable only in the rhizosphere of SP 81-3250 at 60 DAA (Figure 3). This phenomenon shows that for these herbicides, rhizospheric microorganisms were more efficient to use the resources of the environment, in others words, a smaller amount of energy is expended for these microorganisms to complete their life cycle.

The herbicides ametryn and tebuthiron increased the qCO2 of the rhizosphere of SP 81-3250 at 90 DAA (Figure 3). For the rhizosphere of RB867515, the diuron, as well as its mixture with hexazinone, were responsible for increasing the $qCO_2$ values to 90 DAA (Figure 3). Higher values of $qCO_2$ show that there is a higher imbalance in the soil microorganisms, and this effect may reduce soil organic matter values since a more substantial amount of organic carbon is released into the atmosphere as a gas (MASSENSSINI et al., 2015).

The reduction of soil organic matter can compromise cropping system, especially in soils with an intense degradation process, where the organic matter is the component that provides the cation exchange capacity of degraded soils (CIOTTA et al., 2003; SHARMA et al., 2015). These charges retain cationic nutrients such as potassium, nitrogen, calcium, and magnesium, avoiding leaching and gradually providing these nutrients throughout the crop's productive cycle. In addition, higher values of $qCO_2$ elevate the amount of $CO_2$ that is released into the atmosphere, contributing to the increase of the greenhouse effect.

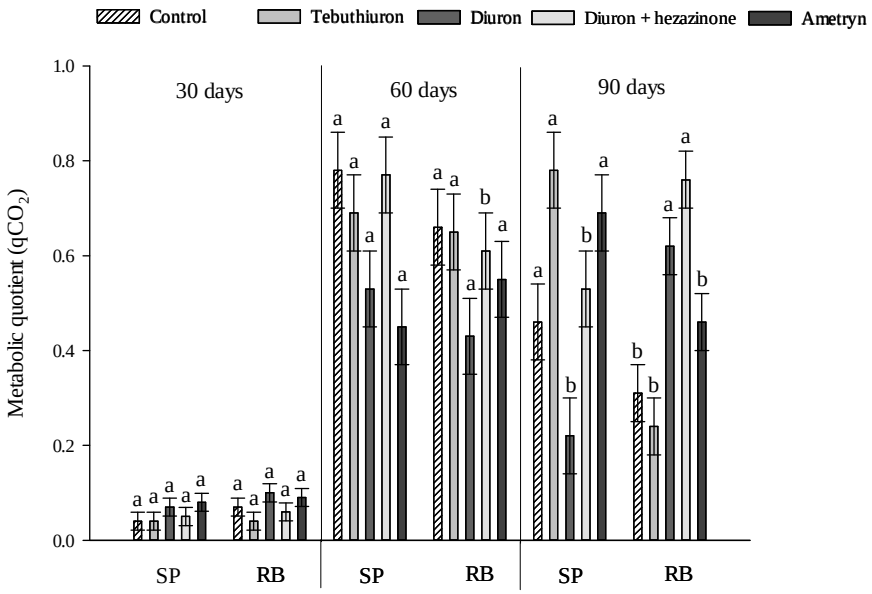


**Figure 3**. Metabolic quotient of the rhizospheric soil of two sugarcane cultivars at 30, 60 and 90 days after herbicide application in pre-emergence. Lowercase letters differ the effect of each cultivar, and error bars differ the effect of each herbicide within the cultivar by the Tukey test at 5% probability.

Mycorrhizal fungi did not colonize sugarcane roots at 30 DAA (Figure 4). For the analysis of the inorganic phosphorus solubilization potential of rhizospheric microorganisms and percentage of roots colonized by mycorrhizal fungi were observed differences among all the treatments (Figure 5). The root colonization by mycorrhizal fungi is a specific interaction, and depends on factors inherent to plant and fungi species (PEREIRA et al., 2013; MASSENSSINI et al., 2013). Also, it is necessary for the microorganism to recognize the plant as a possible host for that symbiosis process occur between them. Therefore, the absence of mycorrhizal colonization in sugarcane cultivars at 30 DAA may be the result of the minimum time required for the fungus to recognize the plant as host (SUN et al., 2015).

Another critical point may be related to the low natural population of spores capable of promoting mycorrhization with sugarcane roots at 30 DAA, making colonization difficult in the initial periods (LEIFHEIT et al., 2014). However, as mycorrhizal fungi colonize roots, a higher amount of spores can be formed, increasing colonization at 60 and 90 DAA.

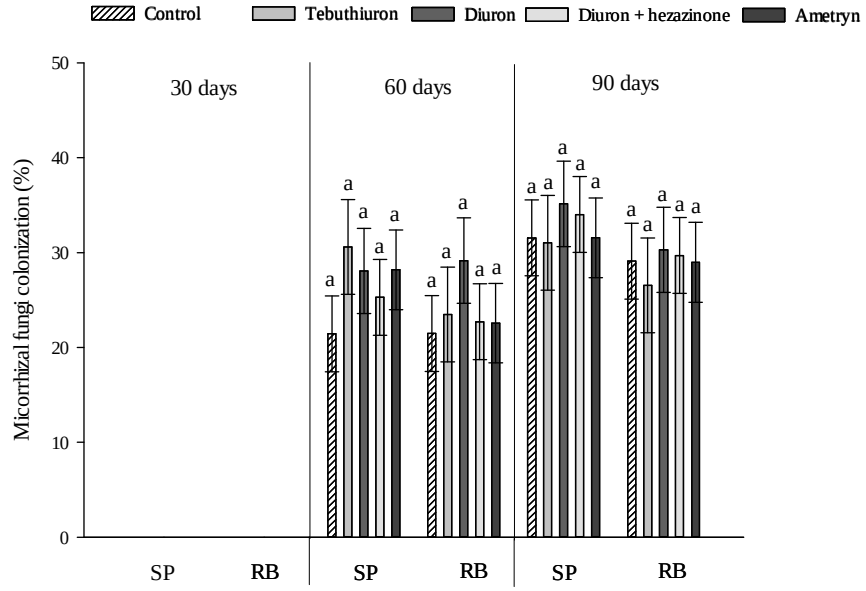


**Figure 4**. Mycorrhizal fungi colonization of the rhizospheric soil of two sugarcane cultivars at 30, 60 and 90 days after herbicide application in pre-emergence. Lowercase letters differ the effect of each cultivar, and error bars differ the effect of each herbicide within the cultivar by the Tukey test at 5% probability.

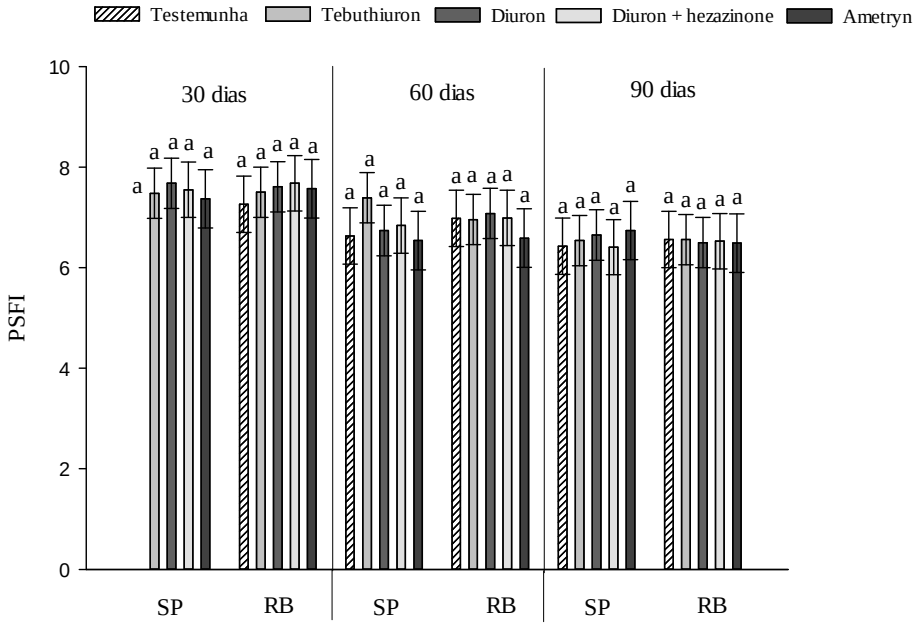


**Figure 5**. Inorganic phosphorus solubilization potential of the rhizospheric soil of two sugarcane cultivars at 30, 60 and 90 days after the herbicide application in pre-emergence. Lowercase letters differ the effect of each cultivar, and error bars differ the effect of each herbicide within the cultivar by the Tukey test at 5% probability.

Therefore, the absence of deleterious effects on colonization by mycorrhizal fungi in RB 867515 and SP 81-3250 cultivars permit the recommendation of the herbicides diuron, tebuthiuron, ametryn, and diuron + hexazinone.

## CONCLUSION

The herbicides did not affect the stability of the rhizosphere sugarcane soil microorganisms 30 days after application. However, at 90 DAH, all herbicides negatively affected sugarcane rhizosphere activity. The rhizosphere of cultivar RB 867515 was the least affected by herbicides.

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