

Microbial activity and population of a red-yellow podzolic soil under organic and conventional cultivation systems of *Citrus sinensis* (L.) Osbeck

Atividade e população microbiana de um podzólico vermelho amarelo sob sistemas de cultivo orgânico e convencional de *Citrus sinensis* (L.) Osbeck

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ABSTRACT

Fruticulture has relevant expression for worldwide plant production. With the expansion of agricultural areas, more attention is given to the soil preservation. Microbial activity is one of the parameters that together with the evaluation of microbial community provide data about soil biological quality. Therefore, the objective of this study was to evaluate the activity and microbial population of a red-yellow podzolic soil cultivated with *Citrus sinensis* under conventional and organic systems. Thus, soil samples were collected in two cultivated areas, under these both systems. A fraction of each soil sample was separated and autoclaved to determine the presence of thermoresistant microorganisms. The microbial activity was measured using the method of capturing CO₂. The microbial population was estimated by selective culture medium, followed by counting of colony forming units. All data was submitted to analysis of variance and covariance ($p \leq 0.05$ and 0.01 respectively). It was observed that, initially, there was a difference in the microbial activity between the cultivation systems, and there was also a high bacterial population. Filamentous fungi and actinomycetes have distinct and peculiar behavior, which were function of the cultivation system and also of the soil samples heating.

Keywords: microbial diversity, soil quality, actinomycetes, fungus, bacteria

RESUMO

A fruticultura tem expressão relevante na produção mundial de plantas. Com a expansão das áreas agrícolas, mais atenção está sendo dada à preservação dos solos. A atividade microbiana é um dos parâmetros que, juntamente com a avaliação da comunidade microbiana, fornece dados sobre a qualidade biológica do solo. Assim, o objetivo deste trabalho foi avaliar a atividade e a população microbiana de um solo podzólico vermelho-amarelo cultivado com *Citrus sinensis* em sistemas convencional e orgânico. Para isso, as amostras de solo foram colhidas em duas áreas cultivadas, uma sob sistema convencional e outra sob sistema orgânico. Uma fração de cada amostra de solo foi separada e autoclavada para se determinar a presença de microrganismos termorresistentes. A atividade microbiana foi medida usando o método de captura de CO₂. A população microbiana foi estimada por meio de cultivo seletivo, seguida pela contagem de unidades formadoras de colônias. Todos os dados foram submetidos a uma análise de variância e covariância ($p \leq 0,05$

e 0,01 respectivamente). Observou-se que, inicialmente, houve uma diferença na atividade microbiana entre os sistemas de cultivo, e também a presença de uma alta população bacteriana. Verificou-se ainda que os fungos filamentosos e actinomicetas têm comportamentos distintos e peculiares em função do sistema de cultivo e também do aquecimento das amostras de solo.

Palavras-chave: diversidade microbiana, qualidade do solo, actinomicetas, fungos, bactérias

INTRODUCTION

Brazilian fruit production is currently diversified when it comes to the cultivated fruit. Citriculture, in turn, had elevated the country to a privileged position, with Brazil being the world's largest producer of this modality (IBGE, 2017). However, several challenges are tackled daily to maintain the productive development of citrines.

For a long time, the world population has been worrying about food quality and safety, thinking about the next generations, which makes it possible to look for better quality foods, also considering the cultivation methods. In this respect, conventional and organic cultivation systems are conceived for citriculture.

The intensification of agricultural production influences an increase of the machines flow in the orchard, where they cause soil damage (Minatel *et al.*, 2006), affecting their physical qualities, resulting in disturbances in the chemical and biological structure, affecting also the fertility. The soil is understood as a living compartment sheltering an infinity of organisms, which contribute directly and indirectly to the fertility, interacting also with the plants.

In conventional crops there is an increase in inputs, which affect the microbial structure of the soil (Sapp *et al.*, 2015), for example through the application of fungicides (Balzergue *et al.*, 2013), which are reported as directly affecting the soil microbiota populations (Zhou *et al.*, 2011; Campos *et al.*, 2015; Wang *et al.*, 2016). In organic cultivation systems there are still some deficiencies regarding plant nutrition, which generates low productivity by productive units. However, it is already known that in these systems there are an interaction and diversity of microorganisms that are capable of increasing the production through microorganism/

plant interaction, such as the presence of arbuscular mycorrhizal fungi (Wang *et al.*, 2012).

There are many mechanisms for inferring about soil quality. Microbial respiration is one of these methods, and is used as a means of inferring microbial activity in crop systems (Barroso *et al.*, 2012), as well as the microbial population (Montaldo *et al.*, 2018). Thus, the objective of this study was to evaluate the respiratory activity and soil microbial population under cultivation of *Citrus sinensis* in conventional and organic systems.

MATERIAL AND METHODS

Experimental area description and soil sampling

The experiment was conducted in two areas of commercial cultivation of *C. sinensis*, one in organic cultivation system (which has organic production certification with the current legislation) and the other in conventional cultivation system (with use of agrochemicals and other inputs). The soil of the region is classified as red-yellow Podzolic (EMBRAPA, 2014). The climate is classified as tropical humid, which is characterized as ideal for the cultivation of fruit plants of tropical and semi-tropical climate.

Soil samples were collected, in both areas, at 20 cm depth from the soil surface, being 10 sub samples in each area. Finally, these sub samples were homogenized, resulting in two composite samples, which were identified and sent to the laboratory for analyzes.

From each soil sample (conventional and organic), half was separated and autoclaved for one cycle of 1h in order to estimate the presence of thermotolerant microorganisms.

Microbiological and chemical analysis

Samples were submitted to microbiological analysis by cultivation on selective and specific culture medium. For this, a serial decimal dilution of the soil samples was performed by diluting 1g of soil in erlenmeyer flasks containing 90mL of saline solution and the dilution was carried out until reaching the fraction 10^{-6} . To determine the soil fungus population, the $\times 10^{-4}$ dilution was used. Aliquots of 1mL of the dilution were inoculated into Petri dishes containing Martin medium (Martin, 1950), which is a selective medium for filamentous fungi and yeasts. The plates were incubated at room temperature for 7 days in the dark and then counting colony forming units per gram (CFU g^{-1}) of soil was performed.

For the isolation of total bacteria and actinomycetes, the media Nutrient Agar (Fahy and Hayward, 1983) and Starch Casein (Kuster and Williams, 1964), respectively, were used. Likewise, the $\times 10^{-6}$ dilution was used for both microorganisms. For total bacteria the Petri dishes were incubated for 3 days and 10 days for the actinomycetes. At the end of the incubation period, plates were submitted to the CFU counts. All the tests were performed with 6 replicates.

The chemical analyzes were carried out at the Soil Physics and Fertility Laboratory of the Federal University of Alagoas.

Microbial activity essay

In order to measure the soil microbial activity, the soil carbon capture method was calculated using the metabolic coefficient, estimated by the C-CO₂ ratio of the basal microbial respiration and the C of the microbial biomass of the samples, according to Anderson and Domsch (1993).

Fractions of 150g of soil were weighed, and transferred to hermetically sealed containers of 1000mL, avoiding the passage of atmospheric air, but allowing respiratory flow to the samples. In each container was placed a smaller one with 10 mL of NaOH solution (1N) to capture CO₂. At seven days intervals, the solutions were removed from the containers and were titrated. Four evaluations

were realized. In the end, 2.5 mL BaCl (0.5 M) was added for precipitation and also 3 drops of the acid-base phenolphthalein indicator. The amount of CO₂ released from the soil was considered after titration of excess NaOH with HCl solution (0.5 M). The calculation of respiration was done using the titration method with CO₂ capture by NaOH by the formula:

$$mEqCO_2 = \frac{(B - V_{am}) \times 1.1 \times 1000}{SW}$$

where:

B = volume of HCl used on titration of white treatment;

V_{am} = volume of HCl used on titration of the samples;

1.1 = conversion factor;

SW = soil weight.

The experiment was composed by 4 treatments: conventional, organic, conventional autoclaved and organic autoclaved, with 6 replicates each.

Statistical analysis

The microbial activity data was submitted to analysis of variance (ANOVA) ($p \leq 0.05$) using the Sisvar software (Ferreira, 2014). The distribution of the microbial population as a function of the microbial activity was subjected to the multivariate analysis by means of the analysis of covariance (ANCOVA) ($p \leq 0.01$) using the software Xlstat®.

RESULTS AND DISCUSSION

The analysis of variance results showed that CO₂ soil evolution in two cultivation systems, expressed in $mEqCO_2 \cdot g^{-1}$, had a decrease in both cultivation systems studied, whether or not they have undergone the autoclaving process. However, at the beginning of the evaluations, it was detected that in the organic cultivation system there was higher microbial respiratory activity (Figure 1).

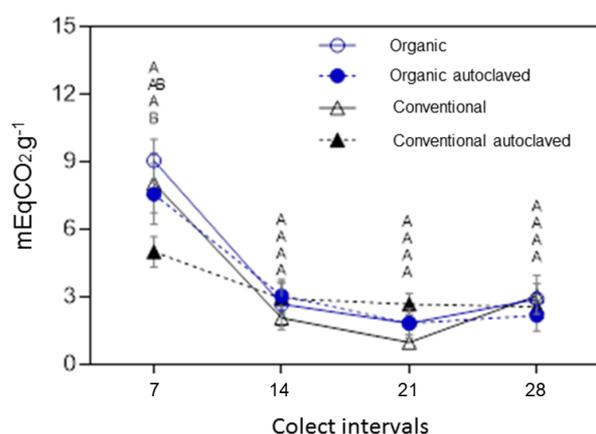


Figure 1 - Microbial activity of a red-yellow Podzolic soil cultivated with *C. sinensis* under conventional and organic systems. Symbols followed by the same letter do not differ between each other by Tukey test ($p \leq 0.05$).

However, the soil microbial activity is a characteristic that has a close relation with the fertility and its management. In this way, it is important to observe the set of factors that converge for this. Thus, soil analyzes (Table 1) show that there are differences in fertility, which are a function of the adopted cultivation system.

CTC rates are mediated by soil organic matter, as they are charged and consequently increased by CTC through nutrient retention, contributing to plant nutrition. Therefore, Montezano *et al.* (2004) refer that management of soil organic matter is critical to improve crop productivity. Thus, it is probable that the organic cultivation system presented greater microbial activity due to the greater maintenance of organic matter, a management that is not adopted in the area where the cultivation is done by conventional methods.

Table 1 - Chemical analysis of a red-yellow podzolic soil under organic and conventional cultivation of *C. sinensis*

Determinations	Conventional	Organic
pH	5.1	5.6
Na (mg/dm ³)	<1	5
P(mg/dm ³)	1	12
K (mg/dm ³)	55	150
Ca (cmol _c /dm ³)	2.14	3.81
Mg (cmol _c /dm ³)	0.60	1.63
Al (cmol _c /dm ³)	0.25	0.00
H + Al (cmol _c /dm ³)	4.13	3.67
CEC ¹ efectiva (cmol _c /dm ³)	3.13	5.84
CEC total (cmol _c /dm ³)	7.01	9.51
V ² (%)	41	61
Organic matter (g/Kg)	17.7	21.6

¹Cations-Exchange Capacity; ²Base saturation on soil.

It is important to take attention to the soil management system, regardless of the cultivation system adopted, ho allows better productivity maintenance and soil quality (Carneiro *et al.*, 2016). Another important aspect is the bases saturation, which is responsible for the increase of the soil pH. In this study it was verified that the organic cultivation system had better balance in its fertility when comparing to the conventional system.

Microbiological analyzes revealed differences between the microbial communities evaluated as actinomycetes and filamentous fungi. However, regardless the crop system, the soil bacterial population remained constant for all evaluated treatments, even with the autoclaving process, $>2 \times 10^8$ CFU g⁻¹ of soil (Table 2). Thus, the bacterial community in red-yellow podzolic soil can be considered a constant population not being affected by the cultivation system.

Table 2 - Microbial population of a red-yellow podzolic soil cultivated with *Citrus sinensis* under organic and conventional systems

Soil	Fungi ²	Actinomycetes ²	Bacteria ²
Organic	3.50x10 ⁴ b	181.66x10 ⁶ a	>2x10 ⁸
Organic autoclaved	0.83x10 ⁴ b	96.83x10 ⁶ ab	>2x10 ⁸
Conventional	21.83x10 ⁴ a	33.33x10 ⁶ bc	>2x10 ⁸
Conventional autoclaved	0.16x10 ⁴ b	2.66x10 ⁶ c	>2x10 ⁸
Pr > F	< 0.0001	< 0.0001	Not significative

¹Means followed by the same letter do not differ between each other by the F test ($Pr \leq 0.01$). ²Values expressed by colonies forming units per g of soil (CFU g⁻¹).

On the other hand, a differentiated behavior was observed between filamentous fungi and actinomycetes showing a population of 33.33×10^4 CFU g^{-1} in conventional cultivation and 181.66×10^4 CFU g^{-1} of soil in organic cultivation, respectively for this second group. Inversely, filamentous fungi population was more representative in conventional cultivation system, showing values of 21.83×10^4 CFU g^{-1} than in organic with values of 3.5×10^4 CFU g^{-1} of soil. When the soil was autoclaved, the microbial population for these microorganisms was reduced. However, for actinomycetes the microbial population still remained considerably higher with a population of 96.83×10^6 CFU g^{-1} in organic soil, while the fungal population was drastically reduced independently of the cultivation system. These results show that actinomycetes have higher resistance to high temperatures and pressure (Figure 2). Thus, filamentous fungi and actinomycetes are groups of microorganisms that have important expression to determine the biological quality of soils as a function of the cultivation system.

When studying soil microbial population, it is important to look at the factors together and not individually. In this way, it is known that the cultivation in organic system has its peculiarities, what differentiates it from the conventional one and makes it better from the ecological point of view. In the conventional cultivation system, for

example, the application of agrochemicals causes imbalance in the microbial community.

So, it is essential the correct agricultural management regardless the system adopted and the crop of interest. Numerous factors contribute to the maintenance of soil fertility, like the population and microbial diversity to be observed, since several species of soil bacteria have the ability to fix atmospheric N and make it available to plants. Fungi, in turn, have the ability to survive in symbiosis with the roots and provide water and nutrients. Both these groups contribute significantly to plant nutrition, acting in addition to mineral fertilization. However, the maintenance of organic matter is of paramount importance, not only for the maintenance of the soil microbiota, but also for the improvement of chemical and physical attributes. In this aspect Spohn (2015) stands out the importance of maintaining organic matter, which is likely to improve the C:N ratio.

Crop rotations, especially those that include cover crops, sustain soil quality and productivity, increasing C, N and microbial biomass, making them a cornerstone for sustainable agroecosystems (McDaniel *et al.*, 2014). On the other hand, in fruit crops of arboreal and perennial species it is difficult to include crop rotations, being necessary the adoption of alternatives that will collaborate with the improvement of the soil quality. Thus, cultivation of legumes can be recommended with subsequent incorporation into the soil.

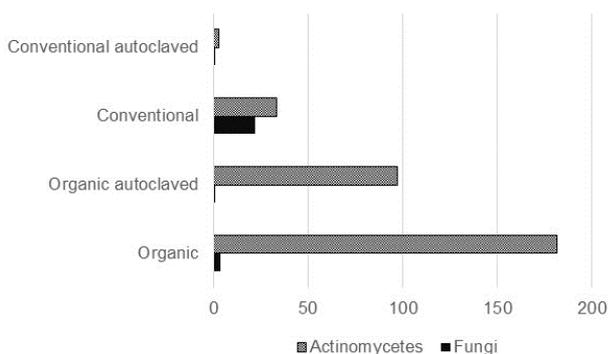


Figure 2 - Population of fungi and actinomycetes expressed in CFU g^{-1} of soil in a red-yellow podzolic soil under organic and conventional cultivation of *C. sinensis*.

CONCLUSIONS

Microbial diversity in soils cultivated with *C. sinensis* showed a high population of bacteria and actinomycetes. In organic cultivation, it is possible to find higher values related to microbial populations, which suffer less impact when submitted to high temperatures. In addition, the microbial community associated with microbial respiratory activity can be adopted as a strategy for the evaluation of biological quality in conventional and organic systems.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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