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# FAMES AND MICROBIAL ACTIVITIES INVOLVED IN THE SUPPRESSION OF CASSAVA ROOT ROT BY ORGANIC MATTER[1]

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**ABSTRACT** - The incorporation of organic matter has been used to manage of diseases caused by soilborne pathogen, but there is a gap in the use of coffee residues on disease supressiveness. The objective of this study was to evaluate the effect of organic matter sources against cassava root rot caused by *F. solani* CFF109. Fertilization with coffee residue (CR), cattle manure (CM), earthworm excrements (EE) and goat manure (GM) resulted in suppression of cassava root rot. The treatments of CR and CM presented higher reduction in the cassava disease severity. There were changes in the soil microbial community structure by organic matter incorporation, mainly in total fungi and Gram-negative bacteria populations. The total organic carbon and magnesium are negatively associated with disease severity. The microbial quotient, alkaline and acid phosphatase activities were positively and the biomarker a-15:0 was negatively associated with disease severity. This study indicated that agro-industrial residues can be recycled for providing organic matter and nutrients with effect for management of plant diseases by suppressing soilborne pathogens. This is the first evidence that the industrial residue of coffee can be use in the management of cassava root rot, caused by *F. solani*.

**Keywords**: *Manihot esculenta. Fusarium solani*. Coffee residue. Extracellular soil enzyme. Microbial community structure.

## FAMES E ATIVIDADES MICROBIANAS ENVOLVIDAS NA SUPRESSÃO DA PODRIDÃO RADICULAR DA MANDIOCA POR MATÉRIA ORGÂNICA

**RESUMO** - A incorporação da matéria orgânica é usada no manejo de doenças causadas por patógenos habitantes do solo, mas existe uma lacuna no uso de resíduos de café na supressão de doenças. O objetivo deste estudo foi avaliar o efeito de fontes de matéria orgânica contra a podridão radicular da mandioca, causada por *Fusarium solani* CFF 109. A fertilização com resíduo de café (CR), esterco bovino (CM), húmus de minhoca (EE) e esterco caprino (GM) resultou na supressão da podridão radicular da mandioca. Os tratamentos CR e CM apresentaram alta redução na severidade da doença em mandioca. Houveram mudanças na estrutura da comunidade microbiana do solo pela incorporação de matéria orgânica, principalmente na população de fungos totais e bactérias Gram-negativa. O carbono orgânico total e magnésio são negativamente associados com a severidade da doença. O quociente microbiano, fosfatases alcalina e ácida foram positivamente e o biomarcador a-15:0 foi negativamente associado com a severidade da doença. Este estudo indica que resíduos agroindustriais podem ser reciclados para fornecer matéria orgânica e nutrientes com efeito para o manejo de doenças de plantas por suprimir patógenos habitantes do solo. Esta é a primeira evidência que o resíduo da indústria de café pode ser usado no manejo da podridão radicular da mandioca, causado por *F. solani.*

**Palavras-chave**: *Manihot esculenta. Fusarium solani*. Resíduo de café. Enzimas extracelulares do solo. Estrutura da comunidade microbiana.

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## INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the most important crops in tropical and subtropical areas because of its importance in the composition of animal and human food. The cassava is also important in the industrial sector because it is one of the five commodities (maize, sugar cane, soy, cassava and oil palm) in bioethanol production and participates in the production of biodiesel and biogas. Moreover, China has been encouraging the increase of the planting areas for bioethanol production from cassava worldwide (NAYLOR et al., 2007).

However, most of the cassava production is from lands of the smallholder farmers, who pay little attention to the physiological and sanitary quality of the cuttings used. The main diseases affecting cassava are mosaic disease, brown leaf spot (*Cercosporidium henningsii* Allesch), white leaf spot (*Phaeoramularia manihotis* (F. Stevens and Solheim) M.B. Ellis), anthracnose (*Colletotrichum gloeosporioides* (Penz.) f. sp. *manihotis*) (MORAIS et al., 2014) and fungi that cause root rot, such as *Phytophthora* spp., *Fusarium solani* (Mart.) Sacco and *Scytalidium lignicola* Pesante (NOTARO et al., 2013).

*Fusarium solani* is least controllable because it can persist in soil via resistance structures and by colonizing non-susceptible hosts. Alternative techniques of plant disease management have researched, such as crop rotation, green manure and soil solarization, are potential techniques to control fungal pathogens. A low cost, accessible, sustainable strategy that causes no damage to the environment is the application of agro-industrial residues to the soil (DIAS et al., 2010). The addition of the residue acts directly in the microbial soil structure, thereby increasing the population of beneficial bacteria and fungi that can inhibit or kill the pathogen in processes involving parasitism, antibiosis and competition; the residue being a source of energy and nutrients that increases microbial activities.

Some studies have assessed the advantages of the use of organic matter sources in relation to the management of plant diseases (LYIMO; PRATT; MNYUKU., 2012). Applications of residues poultry litter and goat manure incorporated to sandy soils was also efficient in suppressiveness against cassava black rot, caused by *S. lignicola*, a soilborne pathogen (SILVA et al., 2013).

The objectives of this study were to: (i) evaluate the effect of organic matter sources added to sandy soils in the suppression of cassava root rot caused by *F. solani* and (ii) identify the influence of these organic matter sources on the soil characteristics, activities and structures (FAMEs) of the soil microbial community.

## MATERIAL AND METHODS

This experiment was conducted in the Regolithic Neosols collected in the dry season from the native forest in a semiarid region of Pernambuco, Brazil. Part of the soil collected was air dried and then passed through a 2-mm sieve before performing chemical and physical analysis. Silva et al. (2013) provided a detailed result of this soil before analysis prior to the addition of organic matter. The soil presented pH ($H_2O$ 1:2.5) = 4.5; P (16.6 mg $Kg^{-1}$); Mg (0.8 cmolc $dm^{-3}$); Ca (0.8 cmolc $dm^{-3}$); Al (0.15 cmolc $dm^{-3}$); Na (0.28 cmolc $Kg^{-1}$); K (0.15 cmolc $Kg^{-1}$) and H + Al (1.8 cmolc $dm^{-3}$). The soil was classified as Entisols.

The cattle (CM) and goat manure (GM), the poultry litter (PL) and earthworm excrements (EE) were collected from producing farms, and the coffee residues (CR) were collected from industry. These sources of organic matter were piled and then subjected to aerobic decomposition.

The isolate *F. solani* CFF109 was obtained from cassava roots showing symptoms of cassava root rot in farms from Pernambuco state, Brazil (8° 42′ 23″ S, 36° 25′ 3″ O) (NOTARO et al., 2013). Inoculum were produced according to Barros et al. (2014) and reactivated throught three subcultures. This isolate was subject to pathogenicity test and showed high virulence. Subsequently, the inoculum was dried, crushed and aliquots of $1 \times 10^6$ conidia $ml^{-1}$ were added to the soil in 3 kg pots each. Then, the cassava plants were covered with bags in order to keep humidity for 48 h. Different organic matter (OM) sources were incorporated into the sandy soil and placed in sterilized plastic pots. The control treatment (CONT) did not receive organic matter.

After three days the *F. solani* inoculums was incorporated into the organic matter, followed by homogenization of the mixture. At 15 days of infestation, cuttings of cassava cv. “Pai Antônio”, measuring 8-10 cm of length with 2-3 buds previously disinfected and dried, were planted in pots individually.

The experimental design was a completely a randomized design with six treatments: control (CONT) soil without organic matter and inoculated with *F. solani*, poultry litter (PL), cattle manure (CM), goat manure (GM), earthworm excrements (EE) and coffee residue (CR), with four replications.

The pots were kept in a greenhouse for a period of 90 days, when it proceeded to evaluate the severity and two samples of 500 g of soil by each repetition were collected. One sample was immediately cooled to 4 °C to determine the biochemical attributes (enzymes activities), the microbial population and the other sample remained at room temperature for the determination of the chemical properties. The experiment was repeated two times.

After 90 days, the evaluation of the severity was performed, based on external symptoms as Barros et al. (2014). The colonization of *F. solani* was identified by morphological characteristics using microscope. Disease severity assessment was recorded using a values scale described by Barros et al. (2014): 0 = no disease, 1 = plants with less than 10% to 25% injuries, 2 = 25% to 50%, 3 = 50% to 75%, 4 = 75% to 100% (dead plants).

Soil pH was determined using an electrode pH meter in water (1:2.5) (v/v). Total organic carbon (TOC) was determined according to the procedure of Yeomans and Bremner (1988). The soil available P was extracted using Mehlich I. Exchangeable $K^+$, $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ were determined. The $Na^+$ and $K^+$ were determined using flame photometry. These results are presented in Table 1.

The soil microbial biomass carbon (MBC) was determined for irradiation method, with the soil samples adjusted to 60% of field capacity. The extraction of the biomass was using $K_2SO_4$ 0.5 M as an extractor. Eighty mL of $K_2SO_4$ 0.5 M was added to each 20 g soil aliquot. The carbon in the extracts was determined using the colorimetric method (BARTLETT; ROSS, 1988).

Soil basal respiration (SBR) was determined by quantifying the carbon dioxide ($CO_2$) released in the process of microbial respiration ($CO_2$ evolution) by the method of adsorption alkaline, with the moisture of the soil samples adjusted to 60% of the capacity field (ANDERSON; DOMSCH, 1985).

Enzyme activities of urease (URE) (EC 3.5.1.5) and phosphatase (acid- Pac and alkaline- Palk) (EC 3.1.3) were quantified based on colorimetric analysis of the products released was incubated with the adequate substrate (Sigma-Aldrich) in standard conditions at 4 °C. The soil URE activity was determined by Kandeler and Gerber (1988) using urea as a substrate. The Pac and Palk activity were determined by Eivazi and Tabatabai (1977) using ρ-nitrophenyl phosphate.

The structure of soil microbial community was determined using the method of quantification and identification of FAMEs reported by Schutter and Dick (2000). The fatty acid nomenclature used was described by Frostegård, Tunlid and Bååth (2011). The numbers of FAMEs detected in soil extract were: i-C15:0, a-C15:0, i-C16:0, i-C17:0 for Gram-positive bacteria (BRADLEY; DRIJBER; KNOPS, 2006; BLAUD et al., 2012). C12:0 2 OH, C12:0 3 OH, C14:0 2OH, C14:0 3OH, C16:1(9)cis, C17:0(9,10)cis, C16:0 2OH, cisC19:0 for Gram-negative bacteria (MERILES et al., 2009; BLAUD et al., 2012). C18:2(9,12)cis, C18:1(9)cis for saprophytic fungi (BRADLEY; DRIJBER; KNOPS, 2006). C14:0, C15:0, C16:0, C17:00, C18:0 Non-specific (BRADLEY; DRIJBER; KNOPS, 2006; BLAUD et al. 2012).

Analysis of variance (ANOVA) was performed to determine the best treatment for reducing the severity of the cassava root rot disease and soil attributes. The means were compared using the Tukey test at $P \leq 0.05$. Fatty acid methyl esters (FAMEs) concentrations of biomarkers were subjected to multivariate of principal component analysis (PCAs) using the data of all treatments. To assess which soil properties were associated with cassava root rot, the linear correlations of Pearson ($P \leq 0.05$) between the disease severity and each of the soil biotic and abiotic attributes were analyzed. The disease severity was confronted with the biotics attributes (FAMEs, Pac, Palk, URE, SBR, MBC, $qCO_2$ and qMic) and abiotics attributes (TOC, pH, P, $Mg^{2+}$, $Ca^{2+}$, $Na^+$ and $K^+$) separately by multivariate of principal component analysis (PCAs) using the data of treatments considered suppressive (goat manure, cattle manure, earthworm excrements and coffee residue) to determine which variables are the most responsible for the differences between treatments.

## RESULTS AND DISCUSSION

The addition of different organic matter sources to sandy soil had different effects ($p \leq 0.05$) regarding the severity of cassava root rot caused by *F. solani* (Figure 1).

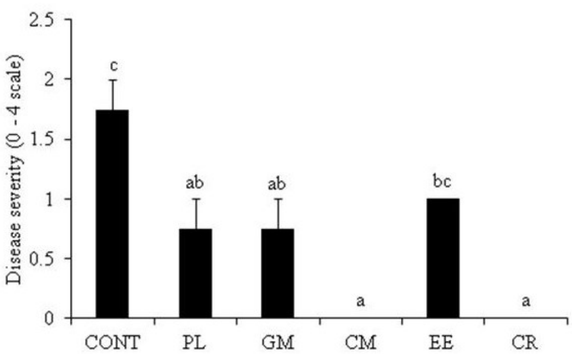


**Figure 1**. Severity of cassava root rot disease in sandy soil with different organic matter sources. CONT = control; PL = poultry litter; GM = goat manure; CM = cattle manure; EE = earthworm excrements; CR = coffee residue. Columns represent average values and error bars represent stand errors (n=4). Different letters above bars indicate significant differences at $P<0.05$ level.

The cassava root rot severity was most reduced in coffee residue (CR) and cattle manure (CM). Cattle manure has been used as a potential means to induce suppressiveness of some diseases, such as gray leaf spot in maize (LYIMO; PRATT; MNYUKU., 2012) and cassava black root (SILVA et al., 2013), because it is an urea-rich organic fertilizer and has higher nutrient contents than those of mineral fertilizer. The organic matter reduces the disease severity by competition among microbial populations, antibiosis, hyperparasitism, systemic acquired resistance and induced systemic resistance, as well as ineffective pathogen proliferation (MEHTA et al., 2014).

The innovation of this work was the use of coffee residues, which presented the best treatment against cassava root rot caused by *F. solani.* The coffee residue can supply nutrients to plants (DIAS et al., 2010), thereby determining the cassava plant to be most resistant to cassava root rot. In addition, the incorporation of organic matter to the soil may generate effect of volatile compounds that kill or reduce viability of fungal structures in the soil. The use of coffee residue can be an excellent management tool of plant diseases, especially for organic and sustainable producers, because it is an industrial waste.

The reductions of disease severity in relation to the CONT treatment were of 98.68% and 96.57% in soils fertilized with CR and CM, respectively. These data indicated that the effect of organic matter sources on the suppressiveness of cassava root rot was directly related to the soil characteristics, as demonstrated by the soil chemical analysis after the experiment (Table 1).

**Table 1**. Properties of organic matter added to sandy soil used in the suppression of cassava root rot, caused by *Fusarium solani.* CONT = control; PL = poultry litter; GM = goat manure; CM = cattle manure; EE = earthworm excrements; CR = coffee residue. Different letters indicate differences at P<0.05 level.

| | | | | Cations ($cmol_c$ $Kg^{-1}$) | | | |
|---|---|---|---|---|---|---|---|
| | TOC (g $Kg^{-1}$) | pH ($H_2O$) | P (mg $Kg^{-1}$) | $Mg^{2+}$ | $Ca^{2+}$ | $Na^{+}$ | $K^{+}$ |
| CONT | 26.0 b | 5.66 c | 0.30 d | 0.43 c | 1.58 d | 0.02 a | 0.01 b |
| PL | 22.7 ab | 6.90 ab | 2.90 b | 0.90 c | 1.10 e | 0.05 a | 0.18 a |
| GM | 31.2 b | 6.82 ab | 2.59 b | 2.48 a | 3.43 c | 0.03 a | 0.12 a |
| CM | 40.5 ab | 7.30 a | 4.97 a | 2.10 b | 3.90 b | 0.04 a | 0.16 a |
| EE | 51.9 a | 7.58 a | 3.51 b | 3.00 a | 7.00 a | 0.04 a | 0.17 a |
| CR | 36.1 ab | 5.87 bc | 1.48 c | 1.05 c | 0.95 e | 0.03 a | 0.06 a |

P = available phosphorus extracted using Mehlich I; Mg = magnesium; Ca = calcium; Na = sodium; K = potassium.

The incorporation of organic matter sources in sandy soil increased TOC content in all treatments compared to control (Table 1). The TOC content was higher in the EE treatment, most likely due to the higher concentration of humic substrate in this material. In a study that used residues as a compost mixed with a clay-loam soil and with crushed bricks (control) the TOC content was also increased considerably, from 0.96 in the control treatment to 23.08 (g 100 $g^{-1}$) in the compost mixture (ONDOÑO; BASTIDA; MORENO, 2014). In contrast, in an another study using the carbon sources plant-derived fresh compost, steer-derived slurry, slurry plus dung and slurry, compost and dung in the suppressiveness of *Fusarium* wilt of flax, no significant correlation between TOC and disease severity was found. However, the authors stated that large variation in TOC content among treatments may have affected the results (SENECHKIN; VAN OVERBEEK; VAN BRUGGEN, 2014).

The CM and CR treatments increased the concentration of TOC in 155.7 and 138.8%, respectively. The literature indicated that the organic soil amendments improve the TOC content and reduced soilborne diseases because the amount of labile carbon present in organic matter are source of energy to microorganisms and can contain antagonistic pathogen microorganisms (JANVIER et al., 2007). These results are consistent with reported studies that used organic matter sources in soilborne diseases because these amendments improve the soil quality, enhance soil microbial biomass and activity and are rich in labile carbon fractions.

The control treatment had the lowest nutrient contents, demonstrating that there was no deficiency in nutrients and that this can improve the cassava health and the soil environment. The nutrients from compost manures are release slowly and provide stable soil nutrient reserve (HEPPERLY et al., 2009).

The incorporation of organic matter sources in sandy soil exhibited greater soil attributes variation, such as SBR and $qCO_2$ (Figure 2a, b). Among the enzymatic activities (Pac, Palk and URE), the one that was sensitive to changes ($p \leq 0.05$) due to the introduction of organic matter sources was the urease activity. An increase in the URE activity was detected in soil with organic matter, mainly in the EE treatment (Figure 2c).

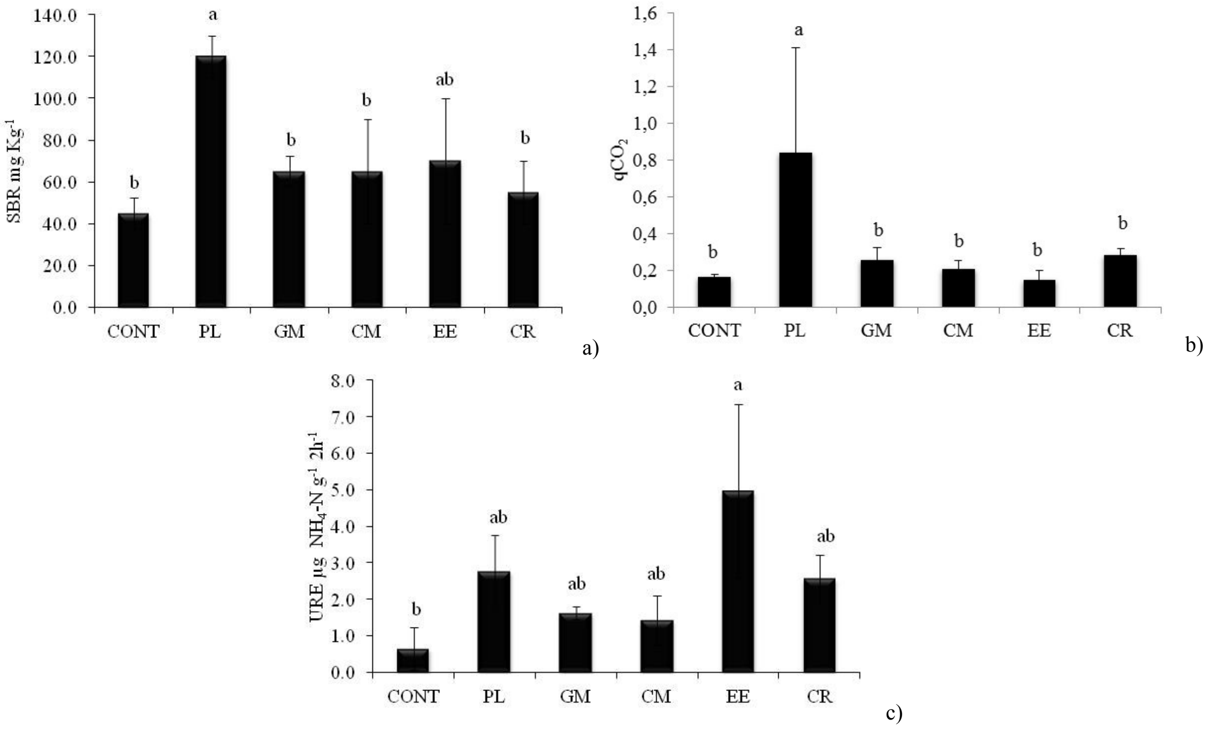


**Figure 2**. Soil basal respiration (a), metabolic quotient- $qCO_2$ (b) and urease activity (c) of soil with different organic matter sources involved in the suppression of cassava root rot caused by *Fusarium solani* CONT = control; PL = poultry litter; GM = goat manure; CM = cattle manure; EE = earthworm excrements; CR = coffee residue. Columns represent average values and error bars represent stand errors. Different letters above bars indicate differences at $P<0.05$ level.

The CONT was the treatment that numerically had the lowest SBR, whereas soils with PL exhibited the largest SBR. Numerous studies indicated that the presence of amino acids, proteins, N, and carbon mineralizable in the PL are responsible for the most microorganism activity. However, the way in which N is available to the plant ($NO_3^-$, $NH_4^+$), the pathogen metabolic requirements and the different ways in which pathogens acquire nutrients can influence the severity of the disease (LYIMO; PRATT; MNYUKU., 2012), as in this study, in which the PL was not efficient in the suppressiveness of cassava root rot.

The microbial biomass carbon (MBC) has been used as a sensitive and early indicator to access the soil quality (MERILES et al., 2009); however, MBC was not an attribute that exhibited change after the introduction of organic matter to sandy soil, nor did the microbial quotient change. The compost amendments can decrease the microbial biomass because the composition of the soil microbial community is modified and the competition among the microorganisms is enhanced (STEINBERG et al., 2004).

The highest $qCO_2$ in the PL treatment indicates that this residue contributed to affect the soil environment by providing disorder in the system. Microorganisms were not efficient to the use organic compounds, resulting in greater release of $CO_2$, thereby forming new microbial cells. This coefficient provides valuable information about microbial use efficiency. The increase in the metabolic quotient was also found when using industrial residues in soils due to the exhaustion of easily available carbon by the intense microbial respiration; this quotient is a biochemical sensor that is amount of substrate mineralized carbon per biomass unity (ONDOÑO; BASTIDA; MORENO, 2014).

There was an increase of the urease activity in all treatments with organic matter. The urease activity in soil with poultry litter and cattle manure was also associated with suppressiveness of cassava root black (SILVA et al., 2013), demonstrating the importance of this attribute, which has been considered one of the most sensitive to detect changes in the soil quality (MEDEIROS et al., 2015). The URE is involved in the N cycle, and the concentration of urease varies considerably with differents organic matter. In contrast, there was no effect of the organic matter addition in Pac and Palk activities. Silva et al. (2013) demonstrate that high Palk activity was associated to increases of severity of cassava black root, caused by *S. lignicola* when PL and goat manure was added to sandy soil. This enzyme are involved in the P cycle, but here, the different P content was not sufficient for demonstrate the differences in Palk and Pac activities. High Pac values are related to the high values of soil TOC and low values of pH and P, when these factors are associated, the activity of this enzyme is favored. In Palk, the change occur as a function of pH alone. The pH could have been responsible for this non-differentiation, since the CR and CONT did not present significant differences as a function of pH and TOC, differing only as a function of P (Table 1).

The FAMEs results indicated that the structure of the microbial community in soil changed among the different organic matter sources as compared to the CONT treatment (Figure 3). The greatest difference was in relation to the group of Gram-negative bacteria, in which only the CONT soils presented the biomarker C14: 0 3 OH.

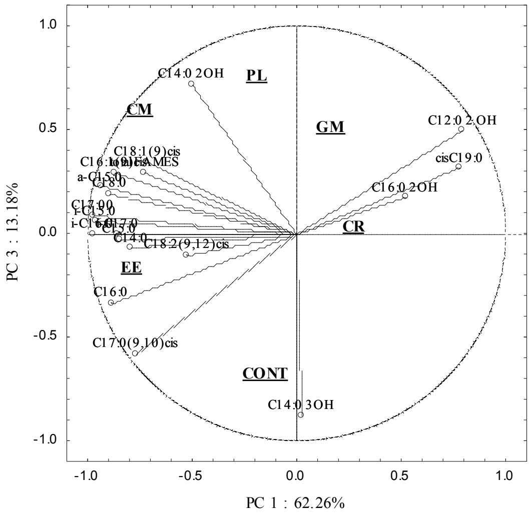


**Figure 3**. Principal components analysis of FAMEs contents in sandy soil with different organic matter sources involved in the suppressivenes and conductiveness of cassava root rot caused by *Fusarium solani* CONT = control; PL = poultry litter; GM = goat manure; CM = cattle manure; EE = earthworm excrements; CR = coffee residue.

The fatty acid methyl (FAMEs) profile results indicated that the CR treatment, which was the most suppressive to cassava root rot, exhibited the highest amount of Gram-negative bacteria, whose biomarker is C12: 0 2 OH. The biomarkers C12:0 2OH and C12:0 3OH is considered as *Pseudomonas* spp., according to Iacobellis et al. (2013). Some studies reported the biological control of *Pseudomonas* against *Fusarium* to be due to antibiosis mechanisms, such as the production extracellular chitinase produced by bacteria which can lyse viable *Fusarium* hyphae, which is indicated as a biocontrol agent of Fusarium wilt in tomato (VELUSAMY; KO; KIM, 2011).

The CM treatment showed higher concentration and diversity of FAMEs, as the C18:1 (9) cis that is indicative of saprophytic fungi (BRADLEY; DRIJBER; KNOPS, 2006). This biomarker may indicate *Trichoderma* spp., but we cannot affirmed because the column used in this study has no specific biomarker. However, *Trichoderma* is a saprophytic fungus used in biological control of plant diseases by different mechanisms. The direct mechanism are mycoparasitism with the chitinase that degrade the pathogen cell, competition and production of antibiotic (MEHTA et al., 2014).

To evaluate the aspects related to the phenomenon of cassava root rot suppressiveness, biotic and abiotic attributes of soil were subjected to linear correlation (Table 2). Among the abiotic factors, TOC and $Mg^{2+}$ content negative correlation with disease severity, indicating that the increase of these elements is favorable to cassava disease management. Among the biotics attributes, the the biomarker a-C15:0 was negatively associated with the disease severity, whereas the increase in Pac, Palk and qMIC were associated with increased cassava root rot disease severity (Table 2).

**Table 2**. Correlations between disease severity and each attributes of soil with different organic matter source involved in suppression of cassava root rot caused by *Fusarium solani.*

| ABIÓTICS | Disease severity |
|---|---|
| Total organic carbon (TOC) | -0.61 [a] |
| pH | -0.40 [ns] |
| P | -0.41 [ns] |
| $Mg^{2+}$ | -0.54 [a] |
| $Ca^{2+}$ | -0.33 [ns] |
| $Na^{+}$ | -0.16 [ns] |
| $K^{+}$ | -0.38 [ns] |
| BIÓTICS | |
| Acid phosphatase (Pac) | 0.61 [a] |
| Alkaline phosphatase (Palk) | 0.82 [a] |
| Urease (URE) | -0.45 [ns] |
| Soil basal respiration (SBR) | 0.28[ns] |
| Microbial biomass carbon (MBC) | -0.04 [ns] |
| Metabolic quotient ($qCO_2$) | 0.13 [ns] |
| Microbial quotient (q*Mic)* | 0.73 [a] |
| C12:0 2 OH | -0.32 [ns] |
| C12:0 3 OH | 0.23 [ns] |
| C14:0 | -0.40 [ns] |
| i-C15:0 | -0.41 [ns] |
| a-C15:0 | -0.54 [a] |
| C15:0 | -0.33 [ns] |
| C14:0 2OH | -0.16 [ns] |
| C14:0 3OH | -0.38 [ns] |
| i-C16:0 | 0.11 [ns] |
| C16:1(9)cis | -0.42 [ns] |
| C16:0 | 0.22 [ns] |
| i-C17:0 | -0.24 [ns] |
| C17:0(9,10)cis | 0.31 [ns] |
| C17:00 | 0.00 [ns] |
| C16:0 2OH | 0.19 [ns] |
| C18:2(9,12)cis | 0.17 [ns] |
| C18:1(9)cis | -0.30 [ns] |
| C18:0 | -0.13 [ns] |
| cisC19:0 | -0.26 [ns] |

a= significant correlation, ns= no significant.

The increased of total organic carbon (TOC) and $Mg^{2+}$ was associated with the decrease of cassava root rot. Higher levels of $Mg^{2+}$ and $K^{+}$ were also associated with lower fungal disease in the take-all disease of wheat because the $Mg^{2+}$ increase the growth of fungi antagonist *Trichoderma viride* Pers. ex Fries and enhanced conidiogenesis and biomass in others hyphomycetes (DUFFY; OWNLEY; WELLER, 1997). This relationship was observed in suppressive and conducive soils against to *Fusarium* wilt in banana (PENG; SIVASITHAMPARAM; TURNER, 1999), corroborating with the present study that used *F. solani*.

The soil TOC has been described as an important factor in the suppressiveness of plant diseases caused by soil borne plant pathogens because the TOC are constantly used by soil microorganisms that compete for this element through several mechanisms. So, when TOC are released after cell lyses, they are soon utilized by microorganisms of rapid growth and metabolism, thereby discouraging the pathogen and favoring competitive saprophytes (BONANOMI et al., 2010).

In relation to the activities of enzymes, the Palk and Pac are associated with suppressiveness. Higher phosphatase activities were also associated with higher cassava black root caused by *S. lignicola* in soil with poultry litter and goat manure (SILVA et al., 2013).

The increase of soil basal respiration and a-C15:0 (Gram positive bacteria) were accompanied by a decrease of the disease severity (negative correlation coefficients), suggesting some mechanisms of bacterial antagonism against plant pathogenic fungi. The population of total bacteria was also responsible for the natural suppressiveness of soils with different cover against cassava root rot caused by *F. solani* (BARROS et al., 2014). Van Beneden et al. (2010) reported that the lignin incorporation in soil may change microbial populations involved in the suppression of *Rhizoctonia solani* in Leest soil, as the concentration the biomarker a-C15:0 that was lower in the control treatment, suggesting that a-C15:0 is an important biomarker involved in suppression of plant disease, as observed in the present study.

The organic matter sources of goat manure (GM), cattle manure (CM), earthworm excrements (EE) and coffee residues (CR) added to sandy soils were considered suppressive to cassava root rot. The data of FAMEs, microbial biomass carbon (MBC), soil basal respiration (SBR) and the activities of phosphatases (Pac and Palk) and urease (URE) of these treatments were considered biotic factors and subjected to multivariate analysis of principal component analysis (PCA), together with the disease severity (Figure 4a).

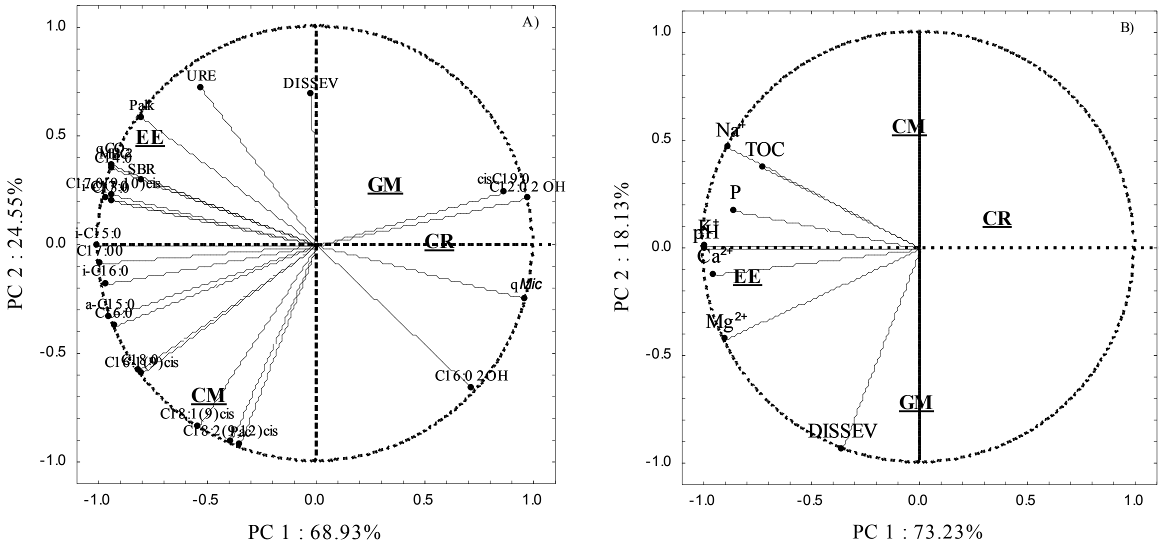


**Figure 4**. Principal components analysis of the (A) abiotic and (B) biotic attributes of the soils with organic matter sources considered suppressive to cassava root rot caused by *Fusarium solani*.

The first principal component (PC1) accounted for 68.93% of the total variation (Figure 4a). The factors that were responsible and that correlated with this component were: i-C15:0> C17:0> C12:0 2OH> i-C17:O= i-C16:0= qMic> C15:0= a-C15:0> qCO2= MBC= C14:0= C17:0 (9.10)cis= C16:0> cisC19:0> C18:0> C16:1(9)cis= SBR= Palk> C16:0 2OH. The second principal component (PC2), the variables Pac> C18:2 (9.12) cis> C18:1 (9) cis> URE > DISSEV accounted for 24.55% of total variation (Figure 4a).

The abiotic factors of the organic matter sources added to the soil exhibited a Cumulative % of 91.36%. All abiotic attributes correlated with PC1, which accounted for 73.23% of the total variation in the data (Figure 4b).

From the multivariate PCA analysis, we demonstrated the importance of FAMEs biomarkers in the separation of treatments considered suppressive to cassava root rot caused by *F. solani*. The FAMEs biomarkers were also the sensitive soil attributes in separate tillage treatments in the soybean growing season (MERILES et al., 2009). In a study using lignin on the suppressiveness of *Rhizoctonia solani*, which is also a fungal inhabitant of soil, Senechkin, Van Overbeek and Van Bruggen (2014) used multivariate PCA using only the FAMEs profile of the microbial community in the soil, obtaining a cumulative 80.8%. In this study, to analyze the organic matter sources on the suppressiveness of cassava root rot caused by *F. solani,* in addition to the biomarkers, the biotic data of soil basal respiration (SBR), biochemical activities (Pac, Palk and URE) and carbon microbial biomass (CBM) were used, which showed a Cumulative% of 93.48%, demonstrating that the suppressiveness phenomenon is better explained when microbial structure and microbial activity are evaluated.

Among the abiotic soil attributes, the pH was the soil attribute that best correlated with PC1. The pH is found to be an indirect factor in explaining certain suppressiveness phenomena in other studies regarding diseases caused by *Fusarium* because the pH can influence the microbial community of soil (SENECHKIN; VAN OVERBEEK; VAN BRUGGEN, 2014).

# CONCLUSIONS

We hypothesize that the structure and activities of the microbial community in soil with *F. solani* would change in response to the addition of organic matter sources, with subsequent reduction in the cassava root rot severity. Our results indicated that the changes were mainly in structure and that some soil parameters are associated with the disease suppressiveness, such as microbial quotient, alkaline and acid phosphatase activities, the FAMEs biomarker a-15:0, total organic carbon and magnesium content. This study produced the first evidence that the residue of the coffee industry can be recycled for use in the ecological management of plant diseases, such as cassava root rot caused by *F. solani*.

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