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EFEITO DO FUNGO MICORRÍZICO ARBUSCULAR E DO VERMICOMPOSTO NA FITORREMEDIAÇÃO DO COBRE EM SOLO ARENOSO

DISSERTAÇÃO DE MESTRADO

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Santa Maria, RS, Brasil

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EFEITO DO FUNGO MICORRÍZICO ARBUSCULAR E DO VERMICOMPOSTO NA FITORREMEDIAÇÃO DO COBRE EM SOLO ARENOSO

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“Se vi mais longe foi por estar de pé sobre ombros de gigantes”

(Isaac Newton)

RESUMO

Dissertação de Mestrado
Curso de Pós-Graduação em Ciência do Solo
Universidade Federal de Santa Maria

EFEITO DO FUNGO MICORRÍZICO ARBUSCULAR E DO VERMICOMPOSTO NA FITORREMEDIAÇÃO DO COBRE EM SOLO ARENOSO

AUTOR: Natielo Almeida Santana
ORIENTADOR: Rodrigo Josemar Seminoti Jacques
CO-ORIENTADOR: Paulo Ademar Avelar Ferreira
Data e local da Defesa: Santa Maria, 12 de setembro, 2014.

Introdução e objetivos: Fungo micorrízico arbuscular e vermicomposto podem reduzir os efeitos deletéricos do cobre às plantas. O objetivo do trabalho foi avaliar o efeito da inoculação do fungo *Rhizophagus clarus* e da adição do vermicomposto de bagaço de uva na fitorremediação por *Canavalia ensiformis* de um solo arenoso com alto teor de Cu. Métodos: Solo foi contaminado com 100 mg Cu kg^{-1} e adubado com cinco doses de vermicomposto para o cultivo de *C. ensiformis*, com e sem a inoculação com *Rhizophagus clarus*. Foram avaliados a disponibilidade de Cu e outros nutrientes no solo e em solução, o acúmulo de Cu e dos outros nutrientes na parte aérea e nas raízes, o crescimento vegetal e a fitotoxicidade do Cu, através da eficiência fotoquímica, da concentração de pigmentos fotossintéticos e da atividade de enzimas do estresse oxidativo. Resultados: A fitoestabilização ocorre melhor na dose de vermicomposto equivalente a 20 mg P kg^{-1} e na presença do *R. clarus*. A fitoextração é maior na dose de vermicomposto equivalente a 40 mg P kg^{-1} e sem a inoculação do *R. clarus*, mas *C. ensiformis* não se apresenta como uma boa planta fitoextratora. Conclusões: O sistema *C. ensiformis*- vermicomposto- *R.clarus* tem potencial de aplicação na fitoestabilização de Cu em solos arenosos.

Palavras-chave: Fitoextração. Fitoestabilização. Resíduo orgânico. Poluição. Feijão de porco.

ABSTRACT

Master's Dissertation
Post-Graduation Program in Soil Science
Federal University of Santa Maria

EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND VERMICOMPOST ON COPPER PHYTOREMEDIATION IN A SANDY SOIL

AUTHOR: Natielo Almeida Santana
ADVISOR: Rodrigo Josemar Seminoti Jacques
DATE AND LOCAL OF THE DEFENSE: Santa Maria, September 12th 2014.

Introduction and objectives: Arbuscular mycorrhizal fungi and vermicompost may decrease the deleterious effects of copper on plants. The goal of the present study was to evaluate the effect of inoculation with the fungus *Rhizophagus clarus* and the addition of grape bagasse vermicompost on phytoremediation by *Canavalia ensiformis* of a sandy soil with high Cu concentration. Methods: Soil was contaminated with 100 mg Cu kg^{-1}, fertilized with five levels of vermicompost, and cultivated with *C. ensiformis* with and without inoculation with *Rhizophagus clarus*. Availability of Cu and other nutrients in the soil and in the soil solution, shoot and root accumulation of Cu and other nutrients, plant growth, and Cu phytotoxicity—using photochemical efficiency, concentration of photosynthetic pigments, and oxidative stress enzyme activities as indicators of Cu phytotoxicity—were evaluated. Results: Phytostabilization showed better performance with the addition of the vermicompost level equivalent to 20 mg P kg^{-1} and in the presence of *R. clarus*. Phytoextraction was higher with the addition of the vermicompost level equivalent to 40 mg P kg^{-1} and without *R. clarus* inoculation. However, *C. ensiformis* was not a good phytoextractor. Conclusions: The system *C. ensiformis*–vermicompost–*R. clarus* exhibited potential for Cu phytostabilization in sandy soils.

Keywords: Phytoextraction. Phytostabilization. Organic fertilizer. Pollution. Jack bean.

SUMÁRIO

1 INTRODUÇÃO GERAL

O Brasil é um dos maiores produtores mundiais de uva (MELLO, 2013) e o estado do Rio Grande do Sul (RS) é responsável por 90% da produção nacional, o que corresponde a cerca de 840.000 toneladas de uva por ano (FLORES; MEDEIROS, 2013). A vitivinicultura gaúcha gera anualmente recursos na ordem de 617 milhões de reais, sendo considerada uma atividade de grande importância à economia do RS (IBGE, 2012). Porém, a expansão da vitivinicultura brasileira também ocorre em outros estados, principalmente em São Paulo, Pernambuco, Paraná, Bahia e Santa Cataria (ALVES; BEZZI, 2013).

No RS, a vitivinicultura iniciou com os imigrantes italianos na região da Serra Gaúcha (MIRLEAN et al., 2009) e se difundiu para outras regiões do estado, como a Encosta Superior da Serra do Nordeste, Planalto Médio, Depressão Central, Alto e Médio Vale do Uruguai, Encosta Inferior da Serra do Nordeste e Campanha Gaúcha (TONIETTO; FALCADE, 2003).

A Serra e a Campanha Gaúcha são consideradas as principais regiões vinícolas do RS, mas apresentam significativas diferenças no perfil da produção. A Serra Gaúcha é a região mais tradicional, onde a viticultura ocorre em pequenas propriedades, com área total média de 15 ha, sendo somente dois hectares destinados para o cultivo da videira. O relevo é acidentado e a mão de obra é familiar (LESSA, 2010). Já a Campanha Gaúcha é a região de expansão da viticultura, onde a atividade é empresarial, com utilização intensiva de capital, realizada em grandes áreas com relevo suave a ondulado (IBRAVIN, 2011). Nesta região, houve um grande crescimento da viticultura nas últimas décadas, principalmente nos municípios de Santana do Livramento, Bagé, Candiota e Hulha Negra (FALCADE, 2006). O clima da região, mesmo sendo mais favorável ao cultivo da videira que a Serra, propicia o aparecimento de importantes doenças fúngicas foliares, que se não controladas adequadamente, reduzem a produtividade (MIRLEAN et al., 2009). Por isso, os produtores utilizam fungicidas cúpricos, principalmente a calda bordalesa (mistura de óxido de cálcio e sulfato de cobre), resultando em uma adição anual considerável de cobre ao solo, pois a maior parte do fungicida tem este ambiente como destino devido à remoção pela água das chuvas e/ou pela senescência das folhas.

Um agravante a esta situação é que muitos vinhedos da Campanha Gaúcha estão localizados sob solos arenosos, que naturalmente apresentam-se ácidos, com baixos teores de argila, de óxidos e de matéria orgânica, o que resulta numa baixa capacidade de sorção do

cobre no solo (MIOTTO et al., 2014). Os relatos de produtores da região informam sobre o menor desenvolvimento das plantas de cobertura e das mudas de videiras estabelecidas sobre áreas de antigos vinhedos, o que pode ser causado pela fitotoxicidade do cobre, presente em elevados teores nestes solos. Além disto, o menor crescimento vegetal nestas áreas aumenta as possibilidades de migração do cobre pela erosão superficial ou lixiviação, o que pode comprometer a qualidade das águas superficiais e subsuperficiais e aumentar a dispersão deste metal pesado nas cadeias tróficas.

Num solo arenoso de vinhedo da Campanha Gaúcha, Girotto et al. (2014) quantificou na camada de 0-20 cm teores totais de cobre de 91 mg kg^{-1} (método H_2O_2 + HF + $HClO_4$), enquanto que no campo nativo adjacente o teor natural era de 14 mg kg^{-1}. Neste sentido, Miotto et al. (2014) também verificou nos primeiros 20 cm de um solo arenoso de um vinhedo teores pseudo-totais (método USEPA 3050B) de 62,5 mg kg^{-1}, sendo que os teores naturais de cobre nestes solos estão próximos a 3,2 mg kg^{-1}. Já Brunetto et al. (2013) observou que os teores de cobre (método 0.01 mol L^{-1} EDTA e 1.0 mol L^{-1} NH_4CH_3COO) estão 70 vezes maiores na camada 0-10 cm e 40 vezes maiores na camada de 10-20 cm que os teores naturais, observados no solo adjacente sem cultivo da videira. Estes resultados indicam que o cultivo da videira por 30 anos elevou drasticamente os teores de cobre nestes solos, o que se caracteriza como um grave problema de poluição ambiental em extensas áreas do Rio Grande do Sul.

Perante isso, alternativas para a remediação destes solos devem ser buscadas como forma de reduzir o risco de contaminação ambiental. Embora os metais pesados sejam considerados tóxicos em elevadas concentrações para maioria das plantas, algumas espécies possuem a capacidade de se desenvolver em ambientes contaminados e acumular estes elementos (DIPU et al., 2012). Tais plantas podem ser utilizadas em programas de fitorremediação, que é uma técnica que utiliza plantas associadas ou não a microrganismos para degradar, remover ou reduzir a toxicidade de poluentes orgânicos ou inorgânicos, como os metais pesados. Pilon-Smits (2005) afirma que a fitorremediação pode englobar as técnicas de fitoextração, fitoestabilização, fitoestimulação, fitovolatilização e fitodegradação. Neste estudo, as técnicas de fitoestabilização e fitoextração foram consideradas. A fitoestabilização consiste na utilização de plantas para estabilizar o metal no solo, reduzindo assim sua movimentação pela erosão e percolação, a exposição aos animais e a probabilidade de entrarem na cadeia alimentar (WONG, 2003). Já a fitoextração baseia-se no uso de plantas para remoção de metais pesados dos solos, mediante a absorção pelas raízes, transporte e concentração na parte aérea, a qual poderá ser coletada e tratada de forma a obter um destino

ambiental correto.

O cultivo de plantas de cobertura em vinhedos é uma alternativa para promover a proteção do solo contra os agentes erosivos, reduzir as variações de umidade e temperatura do solo, aumentar a ciclagem de nutrientes, aumentar o teor de matéria orgânica do solo, estimular a atividade biológica, etc. O Feijão de Porco (*Canavalia ensiformis* (L.) D.C.) é uma leguminosa tropical de crescimento rápido que apresenta capacidade de formar associações mutualísticas com fungos micorrízicos e bactérias fixadoras de nitrogênio. Além disso, o Feijão de Porco tem potencial para ser utilizado em programas de fitorremediação por apresentar capacidade de acumular elevadas concentrações de metais em seus tecidos (VENDRUSCOLO, 2013; RAMIREZ; DUSSAN, 2014), podendo ser cultivado em solos poluídos por cobre após a retirada de antigos vinhedos, a fim de se estabelecer um processo de recuperação destas áreas.

No entanto, normalmente há muitas dificuldades de uma planta fitorremediadora estabelecer-se em um local contaminado devido a elevada fitotoxicidade dos poluentes. A adição de adubos orgânicos ao solo a ser descontaminado pode ser uma alternativa para aumentar a eficiência da fitorremediação (JADIA; FULEKAR, 2008). O vermicomposto é um tipo de adubo orgânico produzido a partir de resíduos que normalmente apresentam-se como problemas ambientais (dejetos de animais, resíduos agrícolas e industriais, etc) e estabilizado pela atuação de minhocas e microrganismos.

A adição de adubos orgânicos estabilizados ao solo pode promover a diminuição da disponibilidade de metais pesados às plantas devido à grande concentração de grupos funcionais com capacidade de ligação a estes metais. Com isto, há um estímulo ao crescimento das plantas nas áreas contaminadas, redução da migração do cobre no solo e favorecimento à fitoestabilização. Por outro lado, a adição de adubos orgânicos cuja composição resulte no aumento das formas solúveis de carbono no solo pode promover a dessorção e maior biodisponibilidade do Cu, o que favorece a fitoextração. Karami et al. (2011) ao usarem um composto orgânico para amenizar o efeito tóxico de Cu e Pb observaram maior produção de matéria seca e também maior absorção destes metais pelas plantas de *Lolium perenne* L.. Os autores atribuem este efeito ao aumento do carbono solúvel no solo, que atuou como um agente quelante, aumentando a disponibilidade dos metais na solução e proporcionando maior disponibilidade de nutrientes para as plantas. Além disto, a produção de adubos orgânicos, como o vermicomposto, também permite dar um destino ambientalmente correto aos resíduos da vinificação (GÓMEZ-BRANDON et al., 2011) Estes apresentam grande disponibilidade na região, como o bagaço de uva, que é gerado numa

proporção de 40% em relação a uva processada pelas vinícolas (ROSSI; SANTOS, 2014). Considerando-se a produção de uva no Rio Grande do Sul, são gerados aproximadamente 336 mil toneladas de bagaço por ano, justificando a utilização do processo de vermicompostagem para redução do volume, estabilização do resíduo e redução do seu potencial poluidor (MARTÍNEZ-CORDEIRO et al., 2013).

A fitorremediação é uma tecnologia relativamente nova e pouco se conhece sobre o efeito dos fungos micorrizícos arbusculares (AMFs) no aumento ou redução da absorção de metais pesados pelas plantas fitorremediadoras. Por um lado, é reconhecido o papel das micorrizas no aumento da absorção de alguns macro e micronutrientes, e por outro, sabe-se que os fungos micorrízicos desempenham um importante papel na proteção das plantas ao excesso de metais pesados, reduzindo a absorção destes pelo sistema radicular (MOREIRA; SIQUEIRA, 2006). Silva et al. (2006) relatam os benefícios da inoculação de AMFs para o crescimento da *Brachiaria decumbens* em solos multicontaminados por metais pesados, sendo este efeito atribuído à redução na concentração destes elementos na parte aérea das plantas micorrizadas. Já Castillo et al. (2011) relataram que a associação simbiótica com AMFs eleva o poder fitoextrator de cobre das plantas de *Tagetes erecta* L.

Devido às informações escassas na literatura referente às interações entre solos arenosos contaminados por cobre, plantas fitorremediadoras, adubos orgânicos e fungos micorrízicos arbusculares, justifica-se a realização de um estudo para testar as seguintes hipóteses:

1) A inoculação do fungo micorrízico *Rhizophagus clarus* e a adição do vermicomposto à base de bagaço de uva no solo arenoso contaminado por cobre possibilita aumentar o teor de cobre na parte aérea e no sistema radicular de *Canavalia ensiformis* (L.) D.C.;

2) A adição de vermicomposto à base de bagaço de uva no solo arenoso contaminado com cobre e a inoculação do fungo micorrízico *Rhizophagus clarus* reduzem os efeitos fisiológicos fitotóxicos do cobre e favorecem o crescimento de *Canavalia ensiformis* (L.) D.C..

Para testar estas hipóteses os objetivos do trabalho foram:

1) Quantificar o efeito da inoculação do fungo micorrízico na absorção de cobre, em parâmetros fisiológicos e no crescimento de plantas de *Canavalia ensiformis* (L.) D.C. em solo arenoso contaminado com cobre;

2) Avaliar o efeito da adição de doses crescentes do vermicomposto derivado do bagaço da uva na absorção de cobre, em parâmetros fisiológicos e no crescimento de plantas

de *Canavalia ensiformis* (L.) D.C. em solo arenoso contaminado com cobre;

3) Avaliar o efeito sinergístico do fungo micorrízico arbuscular e do vermicomposto na fitorremediação do cobre em solo arenoso por plantas de *Canavalia ensiformis* (L.) D.C..

2 ARTIGO I - EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND VERMICOMPOST ON COPPER PHYTOREMEDIATION IN A SANDY SOIL[1]

2.1 Abstract

Introduction and objectives: Arbuscular mycorrhizal fungi and vermicompost may decrease the deleterious effects of copper on plants. The goal of the present study was to evaluate the effect of inoculation with the fungus *Rhizophagus clarus* and the addition of grape bagasse vermicompost on phytoremediation by *Canavalia ensiformis* of a sandy soil with high Cu concentration.

Methods: Soil was contaminated with 100 mg Cu kg^{-1}, fertilized with five levels of vermicompost, and cultivated with *C. ensiformis* with and without inoculation with *Rhizophagus clarus*. Availability of Cu and other nutrients in the soil and in the soil solution, shoot and root accumulation of Cu and other nutrients, plant growth, and Cu phytotoxicity—using photochemical efficiency, concentration of photosynthetic pigments, and oxidative stress enzyme activities as indicators of Cu phytotoxicity—were evaluated.

Results: Phytostabilization showed better performance with the addition of the vermicompost level equivalent to 20 mg P kg^{-1} and in the presence of *R. clarus*. Phytoextraction was higher with the addition of the vermicompost level equivalent to 40 mg P kg^{-1} and without *R. clarus* inoculation. However, *C. ensiformis* was not a good phytoextractor.

Conclusions: The system *C. ensiformis*–vermicompost–*R. clarus* exhibited potential for Cu phytostabilization in sandy soils.

Keywords: Phytoextraction. Phytostabilization. Organic residue. Pollution. Jack bean.

[1] Artigo elaborado segundo as normas da revista Plant and Soil

2.2 Introduction

Soil Cu contamination is a common problem in many agricultural regions worldwide (Andrade et al. 2010). In Southern Brazil, the humid subtropical climate propitiates the occurrence of fungal diseases in vines, and control of such diseases is often performed through frequent application of Bordeaux mixture, causing Cu accumulation in soils (Mirlean et al. 2009). This problem is aggravated by the fact that many vineyards in Brazil are established in sandy soils, with low concentrations of organic matter, resulting in low Cu-sorption capacity and higher environmental contamination potential. Miotto et al. (2014) measured levels of 62.5 mg Cu kg^{-1} at the top 20 cm of a vineyard sandy soil, whereas natural concentrations are 3.2 mg Cu kg^{-1} (USEPA 3050B method). In the same soil, Brunetto et al. (2013) observed Cu concentrations 70 times higher in the 0-10 cm layer and 40 times higher in the 10-20 cm layer compared to adjacent soil without vines (method 0.01 mol L^{-1} EDTA + 1.0 mol L^{-1} NH_4CH_3COO).

Plant cultivation in soils with Cu accumulation normally results in increased heavy metal concentration in plant tissues and higher production of reactive oxygen species (ROS) in plant cells (Briat and Lebrun 1999). ROS, such as the superoxide anion radical (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$) (Kohen and Nyska 2002), cause oxidative damage to lipids, proteins, and nucleic acids. Excess Cu can also inhibit photosynthesis and production of chlorophyll *a* and *b* (Baglyas and Pólos 2014; Cambrollé et al. 2014). As a result of these physiological damages, ground-cover plants and vine seedlings established in old vineyards in Southern Brazil exhibit chlorosis, necrosis, root growth inhibition, and decreased dry matter production (Miotto et al. 2014). This damage results in lower soil cover and increased erosion, Cu contamination of surface water and groundwater (Besnard et al. 2001), and economic losses for wine growers.

However, some plants are able to grow in environments with high soil heavy metal concentrations and to accumulate heavy metals in the shoot and/or roots (Dipu et al. 2012). These plants exhibit enzymatic and non-enzymatic defense mechanisms against the detrimental effects of heavy metal toxicity. Increases in carotenoid production, metal retention on the roots, organic acid complexation, and several changes in cellular metabolism are some of the non-enzymatic defense mechanisms exhibited by these plants (Clemens 1999; Bowler and Fluhr 2000; Resende et al. 2003; Andrade et al. 2009). Jack bean (*Canavalia ensiformis* (L.) D.C.) is a tropical legume, used as a ground-cover crop, and exhibits potential to be used in phytoremediation (Andrade et al. 2010). This species forms symbiotic associations with N-fixing bacteria and arbuscular mycorrhizal fungi (AMF), which increase the capacity of these crops to tolerate several stresses throughout their life cycle (Watts-Williams et al. 2013).

Phytoremediating plants usually have difficulty establishing themselves in soils with high heavy metal concentrations. Phytoremediation efficiency may therefore be increased through mycorrhizal fungi inoculation and addition of organic fertilizers to the soil (Jadia and Fulekar 2008; Fernández-Gómez et al. 2012). Symbiotic associations with arbuscular mycorrhizal fungi were observed to increase the growth of *Tagetes erecta* and Cu phytoextraction in environments with high Cu concentration (Castillo et al. 2011). Moreover, higher Cu extraction by *Oenothera picensis* was observed following addition of organic compounds to the soil (González et al. 2014)

Organic fertilizers can improve the efficiency of phytoremediation. Grape bagasse is a residue of wine production that can be used for production of organic fertilizers. This process represents a more environmentally friendly use of grape bagasse, contributes to the replenishment of part of the nutrients in the vineyard soil, and improves the soils' chemical, physical, and biological properties, especially for sandy soils (Fernández-Bayo et al. 2007). However, an interaction between arbuscular mycorrhizal fungi and vermicompost in the

phytoremediation of a heavy metal-contaminated soil by *Trifolium repens* was not observed (Fernández-Gómez et al. 2012). The synergistic effect of mycorrhizal fungi and vermicompost in phytoremediation may depend on the plant species, fungal species, and the soil and vermicompost characteristics. The goal of the present study was to evaluate the effect of inoculation with *Rhizophagus clarus* and addition of grape-bagasse vermicompost on phytoremediation by *C. ensiformis* of a sandy soil with high Cu concentration.

2.3 Materials and methods

2.3.1 Soil, vermicompost, and plant growth conditions

The soil used to grow the plants was collected from the 0-20 cm soil layer from an area of natural pasture without history of cultivation in a vineyard region in the state of Rio Grande do Sul, Brazil (30°48'27"S and 55°22'42"W) and was classified as Typic Hapludalf (Soil Survey Staff, 2010). The soil was autoclaved to eliminate possible viable spores from native arbuscular mycorrhizal fungi. After 60 days, the soil pH was corrected according to technical recommendations, and 100 mg Cu kg^{-1} soil were added in the form of copper sulfate (33.34%) and copper chloride (66.33%). The soil was incubated for 30 days and analyzed. The results of the analysis were as follows: 140 g kg^{-1} clay (densimeter), 9.0 g kg^{-1} organic matter (Walkley-Black), pH 5.6 (water 1:1), 4.5 mg dm^{-3} P (Mehlich-1), 84.0 mg dm^{-3} K (Mehlich-1), 94.2 mg dm^{-3} Cu (Mehlich-1), 1.6 mg dm^{-3} Zn (Mehlich-1), 44.8% base saturation, and 0% Al saturation. Field capacity was determined in a tension table by saturating the soil samples for 48 hours and subjecting them to 10 kPa for 4 days (Klute, 1986).

Vermicompost was produced from grape bagasse subjected to aerobic composting and

subsequently to vermicomposting with *Eisenia andrei* Bouché (1972) worms. The vermicompost was autoclaved and was chemically analyzed after 60 days (Table 1). Seven days before sowing, a filtrate (without AMF propagules) of non-sterile soil or vermicompost was added to reestablish the native microbial populations in the sterile soil and vermicompost (Haymann and Mosse, 1971).

Canavalia ensiformis (L.) D.C. (jack bean) was grown in a greenhouse (29º41'11.46"S and 53º43'8.28"W). The experimental units consisted of 5-L pots with 3.5 kg soil. The soil moisture was controlled through daily weighings and kept at 70% field capacity by adding distilled water. Five seeds inoculated with *Bradyrhizobium elkanii* were sown on each pot. Thinning was performed eight days following germination, keeping two seedlings per pot.

AMF inoculation was performed using 100 spores of *Rhizophagus clarus* (T.H. Nicolson; N.C. Schenck) C. Walker & A. Schüßler per pot, placed close to the root, following multiplication in trap cultures under laboratory conditions. Spore extraction was performed by wet sieving (Gerdemann and Nicolson, 1963), followed by centrifugation in water at 412 *g* for three minutes and in 45% sucrose (m/v) at 309 *g* for two minutes. Selection and counting of viable spores was performed using a stereomicroscope.

2.3.2 Experimental design

The experimental design was completely randomized, with a 5x2 factorial arrangement and three replicates. Five levels of vermicompost were tested: 0, 4.8, 9.7, 19.4, and 38.8 g vermicompost kg^{-1} soil, supplying 0, 10, 20, 40, and 80 mg P per kg soil (0PV, 10PV, 20PV, 40PV, and 80PV), respectively. Half of the plants of all treatments were inoculated with arbuscular mycorrhizal fungus (+AMF), and the other half were not inoculated (-AMF).Based on a previous experiment, 6.38 mg P kg^{-1} were added as a KH_2PO_4

138 solution to the treatment without vermicompost (0PV) to enable plant growth and production
139 of sufficient dry weight for the analyses.
140
141 2.3.3 Measurements
142
143 The plants were harvested at flowering, 45 days after sowing. Roots were separated
144 from the soil manually and washed in running water, 002 mol L^{-1} EDTA, and distilled water,
145 sequentially. The mycorrhizal colonization rate was estimated based on the presence of
146 hyphae and vesicles in 20 root segments that were 1 cm in length. For this purpose, the root
147 segments were cleared in KOH, stained with 0.05% trypan blue, and mounted onto
148 microscope slides (Giovannetti and Mosse, 1980). Shoots, roots, and nodules were dried in a
149 forced-air oven at 65ºC until a constant weight was reached. Cu, P, K, Mg, Fe, and Zn
150 concentrations in the shoot were determined following digestion with nitro-perchloric acid
151 and using an atomic absorption spectrophotometer (932 AA, GBC, Australia) (Embrapa,
152 1997). Shoot N concentration was determined following digestion with sulfuric acid,
153 according to the Kjeldah1 method.
154 Following harvest, soil Cu, Zn, P, and K were extracted using the Mehlich-1 method,
155 and soil N was determined according to the Kjeldahl method (Bremner and Mulvaney, 1982).
156 A soil solution was obtained using saturation extracts, according to Raij et al. (2001). An
157 aliquot of the soil solution was used for pH determination, and another aliquot was used to
158 determine the copper (Cu^{+2}) and phosphorus (P solution) concentrations using an Inductively
159 Coupled Plasma Atomic Emission Spectrometer (ICP-AES) (Perkin-Elmer, Optima 7000DV,
160 USA).
161 Chlorophyll *a* fluorescence parameters were measured on the fourth fully expanded
162 leaf of one plant per pot at 40 days after seedling emergence, using a pulse-amplitude

163 modulated fluorometer (Junior-Pam, Walz, Germany), between 4:00 a.m. and 6:30 a.m.
164 (dark). Minimal fluorescence (Fo) was measured, following which the leaves were subjected
165 to a saturating light pulse (10 000 μmol m^{-2} s^{-1}) for 0.6 seconds for measuring maximal
166 fluorescence (Fm). Maximum PSII photochemical efficiency (Fv/Fm) was calculated as the
167 ratio of variable fluorescence (Fm-Fo) to maximal fluorescence (Lichtenthaler, 1992).
168 Electron transport rate (ETR_{1500}) was measured through light curves (electron transport rate
169 versus photosynthetically active radiation [PAR]). Light curves were measured by subjecting
170 each sample to nine radiation levels (0, 125, 190, 285, 420, 625, 820, 1150, and 1500 μmol
171 electrons m^{-2} s^{-1}) for 10 seconds.

172 Chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), carotenoids, and beta-carotene
173 concentrations were determined using the same leaf. Leaf samples were collected,
174 immediately frozen in liquid N_2, and stored at -80°C. Next, the samples were ground in liquid
175 N_2, homogenized in 5 mL acetone 80% (v/v), placed in 15-mL tubes, and centrifuged at 4000
176 *g* for 4 minutes at 25°C. Absorbance of the supernatant was read using a spectrophotometer
177 (SF325NM, Bel Engineering, Italy) at 480, 663, 645, 454, and 468 nm. Pigment
178 concentrations were calculated according to Hendry and Price (1993).

179 The shoot samples used for determination of photosynthetic pigments were also used
180 to determine oxidative-stress enzyme activities. The samples were ground in liquid N_2,
181 homogenized in 5.0 mL of 100 mmol L^{-1} potassium phosphate buffer, pH 7.5, containing 1.0
182 mmol L^{-1} EDTA, 3.0 mmol L^{-1} DTT, and 2% PVPP (w/v), and placed in Falcon tubes,
183 according to Azevedo et al. (1998). The samples were centrifuged at 14000 *g* for 30 minutes
184 at 4°C, and a 0.5-mL supernatant aliquot was placed in microtubes and stored at -80 °C.

185 The activity of superoxide dismutase (SOD, EC 1.15.1.1) was determined according to
186 Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount
187 of enzyme that inhibited 50% of the photoreduction of nitro blue tetrazolium (NBT 50%)

188 (Beauchamp and Fridovich, 1971). The activity of non-specific peroxidases (POD, EC
189 1.11.1.7) in the extract was determined according to Zeraik et al. (2008), using guaiacol as
190 substrate and a molar extinction coefficient of 26.6 mmol L^{-1} cm^{-1} (Chance and Maehly,
191 1955). One unit POD was defined as the amount of enzyme that catalyzes the conversion of
192 guaiacol and hydrogen peroxide into 1 μmol tetraguaiacol min^{-1} mL^{-1} extract. Absorbance was
193 measured at 470 nm. Catalase (CAT, EC 1.11.1.6) activity was determined according to
194 Azevedo et al. (1998), using a molar extinction coefficient of 40.0 M^{-1} cm^{-1}. One unit CAT
195 was defined as the amount of enzyme that catalyzes the decomposition of 1 mol H_2O_2 μmin^{-1}
196 mL^{-1} extract. Absorbance was measured at 240 nm.
197
198 2.3.4 Statistical analysis
199
200 The results were subjected to an analysis of variance (ANOVA), followed by a Scott-
201 Knott test, at $p<0.01$. The variables tested were subjected to a principal component analysis
202 (PCA), using the CANOCO 4.5 software (Ter Braak and Smilauer, 1998).
203
204 **2.4 Results**
205
206 2.4.1 Characteristics of the vermicompost and soil
207
208 The nutrient and heavy metal concentrations as well as the pH of the vermicompost
209 produced using grape-bagasse residues were in accordance with the levels recommended in
210 the Brazilian legislation, enabling its addition to the soil as a nutrient source for plants (Table
211 1).
212 Following plant cultivation, the soil pH of all the treatments with vermicompost

addition was higher than 6.4 (Table 2), whereas without vermicompost addition (0PV), the pH was close to the value observed before plant cultivation. The Mehlich-1 extractable P, P in soil solution, N, K, and Zn concentrations increased with increasing levels of vermicompost addition. The opposite was observed for Cu, with the lowest Mehlich-1 extractable Cu and Cu in soil solution concentrations observed at the highest levels of vermicompost addition. A 10 and 70% decrease between treatments 0PV and 80 PV was observed for these last two parameters, respectively. No significant effects of AMF inoculation of *C. ensiformis* plants were observed on the soil chemical parameters (data not shown).

2.4.2 Mycorrhizal colonization and nodulation

No mycorrhizal colonization was observed in the non-inoculated plants. Colonization was lower for treatment 0PV than for the treatments with vermicompost addition (Figure 1a). No significant differences in mycorrhizal colonization were observed between the treatments with vermicompost addition, indicating that although vermicompost addition increased soil P availability, it did not inhibit mycorrhization, even at the highest addition levels tested.

Vermicompost addition resulted in an increase in nodule dry weight up to treatment 40PV (Figure 1b). For treatment 80PV, there was a 14% decrease in nodulation with AMF inoculation and a 31% decrease in nodulation without AMF inoculation, compared to treatment 40 PV. Although the nodule dry weight was low, AMF inoculation resulted in a 121% increase in nodule dry weight at the lowest soil nutrient availability (treatment 0PV).

2.4.3 Shoot growth and nutrient concentrations

Root and shoot dry weight increased with increasing levels of vermicompost addition

238 to the soil (Figure 2). AMF inoculation had a positive effect on shoot dry weight at the lower
239 levels of vermicompost addition, with shoot dry weight being 25 and 27% higher for
240 treatments 0PV and 10PV, respectively, compared to the treatments without AMF inoculation
241 (Figure 2). At the highest levels of vermicompost addition, AMF inoculation decreased shoot
242 dry weight, indicating a parasitic effect of the AMF. There was no effect of AMF inoculation
243 on root dry weight of *C. ensiformis*.

244 The lowest shoot Cu concentrations were observed for the treatments with the highest
245 levels of vermicompost (80PV) (Figure 3a) and exhibiting the highest dry weight (Figure 2),
246 which indicates a dilution effect. For the inoculated plants, no differences in shoot Cu
247 concentrations were observed among the other treatments, although the tendency for a
248 dilution effect was also observed for treatment 40PV. For non-inoculated plants, the highest
249 shoot Cu concentration was observed with the vermicompost level equivalent to 20 mg P kg^{-1}.
250 AMF inoculation decreased the shoot Cu concentration for treatments 20PV and 40PV. When
251 the shoot dry weight was multiplied by the shoot Cu concentration, the level of vermicompost
252 resulting in the highest Cu phytoextraction, and, therefore, the highest total Cu content in the
253 shoot, was the equivalent of 40 mg P kg^{-1} without AMF (Figure 3c).

254 The lowest Cu concentrations and highest root dry weights were again observed in the
255 plants grown with the higher levels of vermicompost tested (Figure 2, 3b), which also
256 indicates a dilution effect. Higher Cu concentrations were observed for the plants with AMF
257 and vermicompost levels equivalent to 10 and 20 mg P kg^{-1}, being significantly different from
258 the Cu levels for the plants without AMF. For the treatments without AMF, higher root Cu
259 concentrations were observed for treatments 0PV and 20PV, with small differences relative to
260 treatment 10PV. When the root dry weight was multiplied by the root Cu concentration,
261 higher Cu phytostabilization was observed for treatment 20PV, being higher for inoculated
262 plants, although this difference was not significant. The higher root Cu concentrations

observed for the AMF treatments at the lower levels of vermicompost (10PV and 20PV) show that mycorrhization tended to increase root Cu concentration and decrease shoot Cu concentration.

Increasing vermicompost addition to the soil resulted in increased shoot P and Mg concentrations and decreased shoot Fe and N concentrations in jack bean plants (Table 3). For the shoot N concentrations, no statistically significant differences among the treatments were observed, but a decreasing trend with increasing vermicompost level was evident. The increase in nodule dry weight with higher vermicompost addition (Figure 1) seems to not have sufficed to avoid the dilution effect on shoot N.

AMF inoculation increased shoot P and Fe concentrations of *C. ensiformis* in most of the treatments tested (Table 3). K and Zn concentrations did not change with the addition of vermicompost or AMF inoculation.

2.4.4 Photosynthetic pigments

The concentration of all the photosynthetic pigments exhibited an overall decrease with increasing vermicompost addition to the soil with Cu (Table 4). This pattern indicates a dilution effect on the leaf photosynthetic pigment concentrations resulting from the higher plant growth at higher levels of vermicompost. Overall, there was no effect of AMF inoculation on the photosynthetic pigment concentrations of *C. ensiformis*.

2.4.5 Photochemical efficiency

Vermicompost addition decreased the effects of Cu phytotoxicity on all the chlorophyll *a* fluorescence parameters (Figure 4). Lower photochemical efficiency was observed for the

plants grown at lower levels of vermicompost (0PV, 10PV, and 20PV), as indicated by higher Fo and lower Y(II), Fv/Fo, and Fv/Fm. A clear trend was not observed for ETR, although this parameter was affected by the treatments tested. AMF inoculation did not improve the chlorophyll *a* fluorescence parameters measured (Figure 4) and only resulted in non-significant increases in PSII effective quantum yield (Y(II)) for all the treatments tested (Figure 4b).

2.4.6 Oxidative-stress enzyme activity

Superoxide dismutase (SOD) activity was significantly higher in the plants grown in soil without addition of vermicompost and, within this treatment, in inoculated plants (Figure 5a). Peroxidase (POD) activity in leaves increased with increasing levels of vermicompost addition, in both the treatments with and without AMF inoculation. POD activity in treatments 20PV, 40PV, and 80PV was on average 20 and 32% higher than in treatments 0PV and 10PV, with and without AMF inoculation, respectively (Figure 5b). AMF inoculation decreased peroxidase activity for all the treatments. No clear trend was observed for catalase (CAT) activity in response to the treatments tested (Figure 5c). However, the highest activity was observed for treatment 80PV. AMF inoculation significantly decreased CAT activity in treatments 0PV, 10PV, and 80PV.

2.4.7 Principal component analysis

The principal component analysis (PCA), considering the variables vermicompost level and AMF inoculation, explained 76.53% of the data variance (Figure 6). Most of the variance in the original data set (61.04%) was explained by the first principal component

(PC1). PC1 was mostly associated with the Cu concentrations in the soil solution, shoot Fe and N concentrations, minimal fluorescence, carotenoids, beta-carotene, chlorophyll *a,* chlorophyll (*a*+*b*), and SOD activity, which were strongly positively correlated. Treatments 0PV and 10PV, with and without AMF inoculation, were related to the Cu concentration in the soil solution, shoot Fe and N concentrations, minimal fluorescence, carotenoids, beta-carotene, chlorophyll *a,* chlorophyll (*a*+*b*), and SOD activity. Treatment 80PV, with or without AMF inoculation, exhibited higher correlation with the shoot dry weight, P in the soil solution, shoot Mg and P concentrations, and PSII quantum yield efficiency (YII). Treatment 40PV, with and without AMF inoculation, was related with the soil solution pH, root and nodule dry weight, POD activity, and maximum quantum yield of photosystem II (PSII) and was also related to the explanatory variables for treatment 80PV. Treatment 20PV was located more to the center of the figure and was related to the shoot Cu concentration without AMF inoculation.

2.5 Discussion

The grape-bagasse vermicompost exhibited P, K, and N concentrations and C/N ratio similar to the vermicomposts produced from wine-production residues used in the studies by Fernández-Bayo et al. (2007) and Paradelo et al. (2011). The quality of the produced vermicompost, and its compliance with the Brazilian legislation, make vermicomposting a cheap and efficient alternative for the treatment of wine-production residues.

Soil Cu availability was decreased by vermicompost addition, especially at the highest levels applied. This process is essential for the phytostabilization of highly contaminated soils, where plant growth may depend on heavy metal complexation by the organic fertilizer and by the plant's rhizosphere (Salt et al. 1995). In contrast, this phenomenon is unfavorable

for phytoextraction, which depends on Cu uptake and translocation into the shoot. The lower Cu availability in the soil solution is attributed to the high affinity of Cu for the vermicompost organic compounds and to the formation of less-soluble complexes in the soil (Jordão et al. 2011; Li et al. 2014). In addition, the increase in soil pH due to vermicompost application resulted in higher Cu complexation by the organic residues added to the soil, thus decreasing Cu bioavailability. The increase in phosphorus availability via vermicompost application may lead to the formation of bonds between the Cu and P in solution, resulting in formation of insoluble phosphates in the soil (Austruy et al. 2014; Elouear et al. 2014).

AMF inoculation had a positive effect on plant growth for the treatments with lower soil phosphorus availability. AMF develop specialized structures for P uptake and transport into the plant, in addition to producing exudates with phosphate-solubilizing activity (Lima and Sousa 2014). Increasing levels of vermicompost addition, although such additions increased the concentrations of Mehlich-1 extractable P and P in the soil solution, did not decrease mycorrhizal colonization. A similar effect was reported by Coelho (2012), who observed that vermicompost applications increased colonization rate of *Annona squamosa* seedlings by *Gigaspora albida*. Contrary to what happened with the application of P in soluble form (K phosphate), where mycorrhizal colonization was significantly reduced.

For the treatments with lower levels of vermicompost addition, the decrease in plant growth resulted in lower nodule dry weight. The plant nutritional deficiency causes changes in nutrient allocation within the root, prioritizing growth in detriment to the biological N fixation (Kleinert et al. 2014). Nodule dry weight decreased in treatment 80PV due to the high soil N concentration (Zahran, 1999). This result is in accordance with Lobo et al. (2012), who observed that N levels higher than 140 mg N kg^{-1}, applied to the soil via organic compost, decreased nodule dry weight in soy beans.

The highest biomass production was observed at the highest level of vermicompost.

363 However, due to the lower Cu availability in the soil solution, the phytoextracted or
364 phytostabilized Cu content was lower. Greater Cu availability in soil solution, and
365 consequently higher Cu concentration in the tissues, was observed for the treatments without
366 vermicompost addition and at the lowest level of vermicompost tested. However, the lower
367 plant growth resulted in low Cu concentration in the root and shoot. The most favorable
368 conditions for phytoextraction were observed for treatment 40PV, and those for
369 phytostabilization were observed for treatment 20PV, which exhibited a combination of high
370 shoot biomass production and root Cu accumulation. In these treatments, AMF inoculation
371 decreased shoot Cu concentration, showing that the use of AMF for Cu phytoextraction by *C.*
372 *ensiformis* is not recommended.

373 Vermicompost addition to the soil also decreased the Fe uptake. This pattern may have
374 resulted from Fe chelation, complexation, adsorption by the vermicompost organic
375 compounds, or immobilization by the soil microbiota influenced by the vermicompost
376 (Fernadéz-Gómez et al. 2012). In contrast, the shoot Fe concentration was significantly higher
377 in the mycorrhizal plants. This result is in accordance with Sandhya et al. (2014), who
378 observed that *Glomus mossae* inoculation increased shoot Fe concentration in *Marsdenia*
379 *volubilis* due to phenol and organic acid exudation by the fungus, which promote the
380 formation of soluble complexes and increase Fe diffusion to the root. Thus, mycorrhizae are
381 believed to play an important part on the increase of Fe uptake by plants fertilized with
382 organic materials because these mycorrhizae decrease Fe availability and uptake.

383 Carotenoid concentrations were higher without vermicompost addition and at the
384 lowest vermicompost levels tested. Increased carotenoid production has also been observed in
385 *Carthamus tinctorius* subjected to high Cu concentrations and has been attributed to a non-
386 enzymatic antioxidant response of plants subjected to stress (Ahmed et al. 2010). Carotenoids
387 possess a phytoprotective and phytoregulatory function in photosynthetically active plants

388 (Giuliano 2014). For the remaining photosynthetic pigments, a dilution effect may have
389 occurred in the treatments with high vermicompost levels because chlorophyll concentrations
390 were higher for the plants with lower dry weight. A dilution effect was also reported by Guo
391 et al. (2014) for phytoremediation of zinc, arsenic, lead, and cadmium by *Lolium multiflorum*
392 following addition of different soil amendments.

393 Excess Cu in plant tissues resulted in detrimental effects for the photosynthetic
394 machinery, as indicated by the chlorophyll fluorescence parameters (González-Mendoza et al.
395 2013). Increases in minimal fluorescence (Fo) result from the destruction of photosystem II
396 (PSII) reaction centers or decreased transfer of excitation energy to the reaction center caused
397 by excess Cu in the plant tissues (Baker and Rosenqvst, 2004). Cu toxicity causes degradation
398 of the chloroplast's internal content and substitution of Mg by Cu in chlorophylls, and
399 decreased PSII maximum quantum yield (Fv/Fm) and effective quantum efficiency (Y(II))
400 due to Cu toxicity have been previously reported (Cambrollé et al. 2015). Plants exhibiting
401 Fv/Fm close to 0.85, with some variation for different plant species, are considered healthy
402 (Kalaji 2008). However, much lower values indicate stress conditions, which decrease the
403 PSII photochemical capacity. This pattern was observed for the treatment without
404 vermicompost addition, for which excess Cu and nutritional deficiency, especially of P,
405 decreased Fv/Fm to 0.67 in the absence of AMF. This result indicates that the use of
406 vermicompost at adequate levels is excellent for the amelioration of Cu toxicity effects on
407 chlorophyll *a* fluorescence, which is another beneficial effect of vermicompost for
408 phytoremediation.

409 In addition to non-enzymatic defense components, such as carotenoids, cells produce a
410 range of antioxidant enzymes in response to ROS formation, which is higher in the presence
411 of high Cu concentrations (Barbosa et al. 2014). Higher SOD activity was observed for the
412 treatment without vermicompost addition, indicating an ameliorating effect of vermicompost

413 on ROS production in *C. ensiformis*. SOD is the first defense enzyme against ROS production
414 in the metabolism, and its activity is induced in the presence of heavy metals in plants (Zhang
415 et al. 2007). Fe is a SOD cofactor (M'Sehli et al. 2014), and SOD activity was higher in the
416 plants with higher Fe uptake.

417 POD activity was decreased by mycorrhization and vermicompost addition at all the
418 levels tested, indicating lower ROS production in mycorrhizal than in non-mycorrhizal plants.
419 Similar results have been reported for *Tagetes erecta* plants subjected to high levels of
420 cadmium and inoculated with AMF (Liu et al. 2011).

421 Cu contamination of a sandy soil with low clay and organic matter contents resulted in
422 high available Cu concentrations in the solid and liquid soil phases. Addition of vermicompost
423 decreased the soil Cu concentrations and resulted in higher mycorrhizal colonization, nutrient
424 uptake, and plant growth. The high biomass production resulted in dilution of shoot Cu
425 concentrations and therefore in lower damages to the photosynthetic machinery caused by
426 ROS production. As a result, phytostabilization and phytoextraction improved significantly at
427 the vermicompost levels equivalent to 20 and 40 mg P kg^{-1}, respectively.

428 AMF inoculation resulted in higher biomass production of the plants grown at lower
429 soil nutrient availability. In addition, the arbuscular fungus increased shoot P concentrations
430 for the treatments with high vermicompost levels and significantly increased Fe uptake by
431 plants. In contrast, AMF inoculation decreased shoot Cu concentration. This result indicates
432 that inoculation should be used in phytostabilization programs but not in phytoextraction
433 ones. Further studies are needed, but the present results show a significant effect of the fungus
434 on the decrease of peroxidase activity, which may indicate lower ROS production by
435 mycorrhizal plants.

436 Vermicompost addition to the soil resulted in increased shoot Cu concentration of *C.*
437 *ensiformis*, especially at the vermicompost level equivalent to 40 mg P kg^{-1}. This result shows

438 that vermicompost may be used to increase the efficiency of Cu phytoextraction in
439 contaminated soils, although *C. ensiformis* did not exhibit characteristics typical of
440 accumulator plants, which accumulate more than 100 mg Cu kg^{-1} at the shoot (Boyd 2007).
441 Thus, *C. ensiformis* is best indicated for phytostabilization programs. The present results
442 indicate that a potential phytostabilization program in Cu-contaminated areas may be
443 established using *C. ensiformis* inoculated with the AMF *R. clarus* and fertilized with
444 vermicompost.
445

446 **2.6 Conclusions**

447

448 Phytostabilization of a Cu-rich soil by *C. ensiformis* was increased by the addition of
449 vermicompost levels equivalent to 20 mg P kg^{-1} and inoculation with the mycorrhizal fungus
450 *R. clarus*. Under the tested conditions, phytoextraction was increased with vermicompost
451 addition to the soil at levels equivalent to 40 mg P kg^{-1} and without inoculation with the
452 mycorrhizal fungus *R. clarus*. The system *C. ensiformis*–vermicompost–*R. clarus* has
453 potential for application in Cu phytostabilization of sandy soils.
454

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Tables

Table 1 Chemical parameters of grape-bagasse vermicompost and limits established by the Brazilian legislation.

Parameters	Units	Vermicompost	Limits[5]
Nitrogen[1]	g kg^{-1}	32.0	min. 5.0
Phosphorus[2]	g kg^{-1}	3.0	-
Potassium[2]	g kg^{-1}	12.0	-
Carbon[1]	g kg^{-1}	346.0	min. 150.0
C/N ratio	-	10.5	max. 14.0
pH	-	8.0	min. 6.0
Mercury[3]	mg kg^{-1}	< 0.01	max. 0.4
Copper[4]	mg kg^{-1}	39.0	max. 70
Zinc[4]	mg kg^{-1}	27.2	max. 200
Cadmium[4]	mg kg^{-1}	< 0.2	max. 0.7
Nickel[4]	mg kg^{-1}	4.0	max. 25
Chrome[4]	mg kg^{-1}	7.0	max. 7.0
Lead[4]	mg kg^{-1}	3.0	max. 45
Molibdenum[4]	mg kg^{-1}	0.2	-

[1]Determined using an Elemental Analyzer (Flash 1112, Thermo Finnigan, Italy).[2]Acid digestion in sulfuric acid and determination in Atomic Absorption Spectrophotometer (AAS) (GBC, 932 AA, USA), according to EMBRAPA (1997). [3]Method EPA7471A. [4]Method EPA3050.[5]Normative instruction n° 46, 10/06/2011, MAPA (BRAZIL, 2011).

Table 2 Soil chemical characteristics following addition of 100 mg Cu kg^{-1} and cultivation of *C. ensiformis* for 43 days with different levels of grape-bagasse vermicompost.

Parameters	Treatments					
	0PV	**10PV**	**20PV**	**40PV**	**80PV**	**CV (%)**
pH solution	5.78 b[(1)]	6.52 a	6.48 a	6.51 a	6.45 a	1.42
P Mehlich-1 (mg kg^{-1})	3.46 e	6.16 d	9.25 c	13.70 b	25.58 a	8.18
P solution (mg L^{-1})	2.18 c	5.77 b	7.58 b	6.56 b	15.32 a	12.88
N total (mg kg^{-1})	7.43 c	11.08 c	12.22 c	76.56 b	108.07 a	18.03
K Mehlich-1 (mg kg^{-1})	73.20 c	81.60 c	85.20 c	144.00 b	193.20 a	17.04
Zn Mehlich-1 (mg kg^{-1})	2.05 c	3.70 b	4.53 a	5.48 a	5.96 a	15.15
Cu Mehlich-1 (mg kg^{-1})	81.70 a	77.26 b	76.53 b	77.43 b	73.58 c	2.15
Cu solution (mgL^{-1})	2.83 a	1.25 b	1.14 b	0.95 c	0.84 c	8.29

[(1)]Means followed by the same letter within the same row were not significantly different according to the Scott-Knott test (p<0.01).

Table 3 Effect of increasing levels of vermicompost addition (0PV, 10PV, 20PV, 40PV, and 80PV) and AMF inoculation on the shoot P, N, K, Mg, Fe, and Zn concentrations of *C. ensiformis* grown in a sandy soil to which 100 mg Cu kg^{-1} were added.

	Inoculation	**0PV**	**10PV**	**20PV**	**40PV**	**80PV**
P (mg kg^{-1})	-AMF	126.5Ac[(1)]	158.0Ab	109.0Bd	165.8Bb	356.8Ba
	+AMF	137.7Ad	151.8Ad	176.8Ac	274.7Ab	391.3Aa
N (g kg^{-1})	-AMF	11.82^{ns}	12.43^{ns}	10.22^{ns}	8.70^{ns}	7.52^{ns}
	+AMF	11.85^{ns}	11.61^{ns}	10.25^{ns}	7.87^{ns}	7.48^{ns}
K (g kg^{-1})	-AMF	13.50Ab	17.90Aa	13.50Ab	13.59Ab	13.60Bb
	+AMF	11.37Ab	15.11Ab	13.90Ab	14.35Ab	18.97Aa
Mg (g kg^{-1})	-AMF	26.63Ab	23.98Ab	28.44Aa	30.23Aa	31.84Aa
	+AMF	28.24Ab	25.20Ab	26.82Ab	28.23Ab	35.91Aa
Fe (mg kg^{-1})	-AMF	37.20Ba	28.30Ab	24.30Bb	13.40Bc	11.11Bc
	+AMF	47.82Aa	29.00Ab	31.70Ab	20.50Ac	17.42Ac
Zn (mg kg^{-1})	-AMF	4.70^{ns}	3.76^{ns}	3.40^{ns}	4.70^{ns}	3.93^{ns}
	+AMF	4.40^{ns}	3.70^{ns}	5.13^{ns}	4.26^{ns}	3.86^{ns}

[(1)]Means followed by the same uppercase letter (effect of AMF inoculation for each level of vermicompost) and lowercase letter (effect of vermicompost levels for treatments with and without AMF inoculation) were not different according to the Scott-Knott test. ns, non-significant (F test; $p<0.01$).

Table 4 Effect of increasing levels of vermicompost addition (0PV, 10PV, 20PV, 40PV, and 80PV) and AMF inoculation on the chlorophyll *a*, chlorophyll *b*, total chlorophyll (*a*+*b*), carotenoids, and beta-carotene concentrations (mg g^{-1} fresh weight) of leaves of *C. ensiformis* grown in a sandy soil to which 100 mg Cu kg^{-1} were added.

		Treatments				
	Inoculation	**0PV**	**10PV**	**20PV**	**40PV**	**80PV**
Chl *a*	-AMF	1.10 Aa[(1)]	1.06 Aa	0.71 Bb	0.77 Ab	0.65 Ac
	+AMF	1.10 Aa	1.01 Aa	0.96 Aa	0.59 Bc	0.81 Ab
Chl *b*	-AMF	0.44 Ac	0.69 Aa	0.28 Ad	0.29 Ad	0.58 Ab
	+AMF	0.42 Ab	0.70 Aa	0.38 Ac	0.22 Ad	0.30 Bd
chl (*a*+*b*)	-AMF	1.54 Aa	1.76 Aa	0.99 Bb	1.03 Aa	1.21 Aa
	+AMF	1.52 Ab	1.71 Aa	1.34 Ac	0.81 Bd	1.11 Ad
Carot.	-AMF	0.33 Aa	0.31 Aa	0.25 Ab	0.20 Ab	0.21 Ab
	+AMF	0.33 Aa	0.32 Aa	0.29 Aa	0.20 Ab	0.22 Ab
b-carotene	-AMF	0.56 Aa	0.55 Aa	0.41 Ab	0.34 Ab	0.37 Ab
	+AMF	0.54 Aa	0.55 Aa	0.49 Aa	0.33 Ab	0.36 Ab

[(1)]Means followed by the same uppercase letter (effect of AMF inoculation for each level of vermicompost) and lowercase letter (effect of vermicompost levels for treatments with and without AMF inoculation) were not different according to the Scott-Knott test. ns, non-significant (F test; $p<0.01$).

Figure Legends (figures were prepared using the SigmaPlot 11.0 software)

Fig.1 Arbuscular mycorrhizal colonization (a) and nodule dry weight (b) in *C. ensiformis* plants inoculated or not with AMF. The plants were grown for 45 days in a sandy soil to which 100 mg Cu kg^{-1} were added and with different levels of grape bagasse vermicompost addition. Letters indicate differences between vermicompost levels within each inoculation treatment. Means followed by the same letter were not significantly different according to the Scott-Knott test. ns, non-significant; ** significant effect of AMF inoculation within each level of vermicompost (F test; $p<0.01$).

Fig. 2 Shoot and root dry weight of *C. ensiformis* plants inoculated or not with AMF. The plants were grown for 45 days in sandy soil to which 100 mg Cu kg^{-1} were added with different levels of grape bagasse vermicompost addition. Letters indicate differences between vermicompost levels within each inoculation treatment. Means followed by the same letter were not significantly different according to the Scott-Knott test. ns, non-significant; **significant effect of AMF inoculation within each level of vermicompost (F test; $p<0.01$).

Fig. 3 Cu concentration at the shoot (a) and roots (b) and accumulated Cu concentration per pot in the shoot (c) and roots (d) of *C. ensiformis* inoculated or not with AMF. The plants were grown for 45 days in sandy soil to which 100 mg Cu kg^{-1} soil were added with different levels of grape bagasse vermicompost. Letters indicate differences between vermicompost levels within each inoculation treatment. Means followed by the same letter were not significantly different according to the Scott-Knott test. ns, non-significant; **significant effect of AMF inoculation within each level of vermicompost (F test; $p<0.01$).

Fig. 4 Minimal fluorescence (Fo) (a), PSII effective quantum yield efficiency (Y(II)) (b), variable fluorescence/minimal fluorescence ratio (Fv/Fo) (c), PSII maximum quantum yield (Fv/Fm) (d), and electron transport rate at the highest radiation (ETR_{1500}) (e) for *C. ensiformis* plants inoculated or not with AMF. The plants were grown for 45 days in sandy soil to which 100 mg Cu kg^{-1} soil were added and at different levels of grape bagasse vermicompost. Letters indicate differences between vermicompost levels within each inoculation treatment. Means followed by the same letter were not significantly different according to the Scott-Knott test. ns, non-significant; **significant effect of AMF inoculation within each level of vermicompost (F test; $p<0.01$).

Fig. 5 Superoxide dismutase (SOD) (a), peroxidase (POD) (b), and catalase (CAT) (c) activity in *C. ensiformis* inoculated or not with AMF. The plants were grown for 45 days in sandy soil to which 100 mg Cu kg^{1} soil were added at different levels of grape bagasse vermicompost. Letters indicate differences between vermicompost levels within each inoculation treatment. Means followed by the same letter were not significantly different according to the Scott-Knott test. ns, non-significant; **significant effect of AMF inoculation within each level of vermicompost (F test; $p<0.01$).

Fig. 6 Principal component analysis (PCA) for shoot copper (Cu-shoot), iron (Fe-shoot), magnesium (Mg-shoot), nitrogen (N-shoot), and phosphorus (P-shoot) concentrations; root copper (Cu-root); soil solution Cu^{2+} (Cu_{solut}), phosphorus (P_{solut}), and pH; shoot (Shoot), root (Root), and nodule (Nodule) dry weight; superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activity; minimal fluorescence (Fo), PSII maximum quantum yield (Fv/Fm), PSII effective quantum efficiency (YII), and electron transport rate at the highest radiation (ETR); and chlorophyll *a*, chlorophyll *b*, chlorophyll (*a*+*b*), carotenoids, and β-

carotene concentrations in *C. ensiformis*, inoculated or not with the arbuscular mycorrhizal fungus. The plants were grown for 45 days in a sandy soil to which 100 mg Cu kg^{-1} soil were added and at different levels of grape bagasse vermicompost.

Fig.1

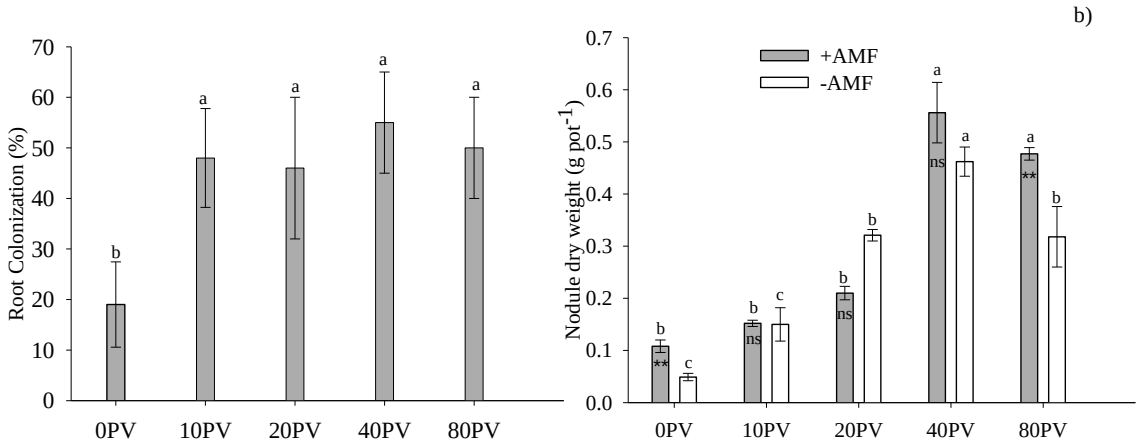

b)
Root Colonization (%)
70
60
50
40
30
20
10
0
a
a
a
a
b
0PV
10PV
20PV
40PV
80PV
Nodule dry weight (g pot-1)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
+AMF
-AMF
b
**
c
b
ns
c
b
ns
b
a
ns
a
a
**
b
0PV
10PV
20PV
40PV
80PV

Fig.2

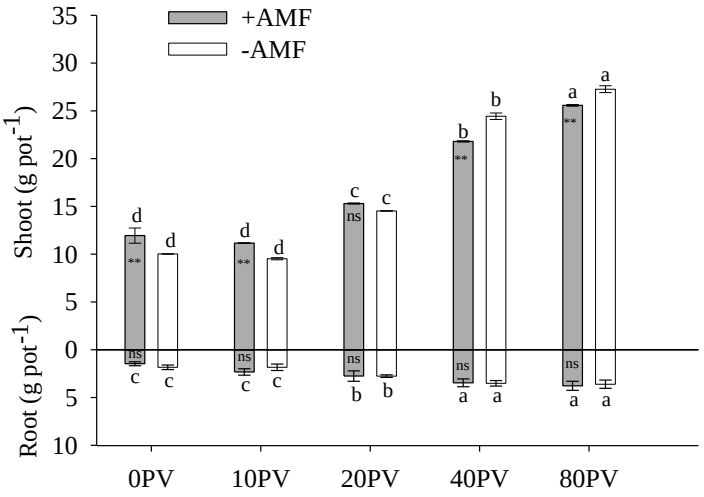

+AMF
-AMF
Shoot (g pot-1)
Root (g pot-1)
35
30
25
20
15
10
5
0
5
10
0PV
10PV
20PV
40PV
80PV
d
d
d
d
c
c
b
b
a
a
**
ns

Fig. 3

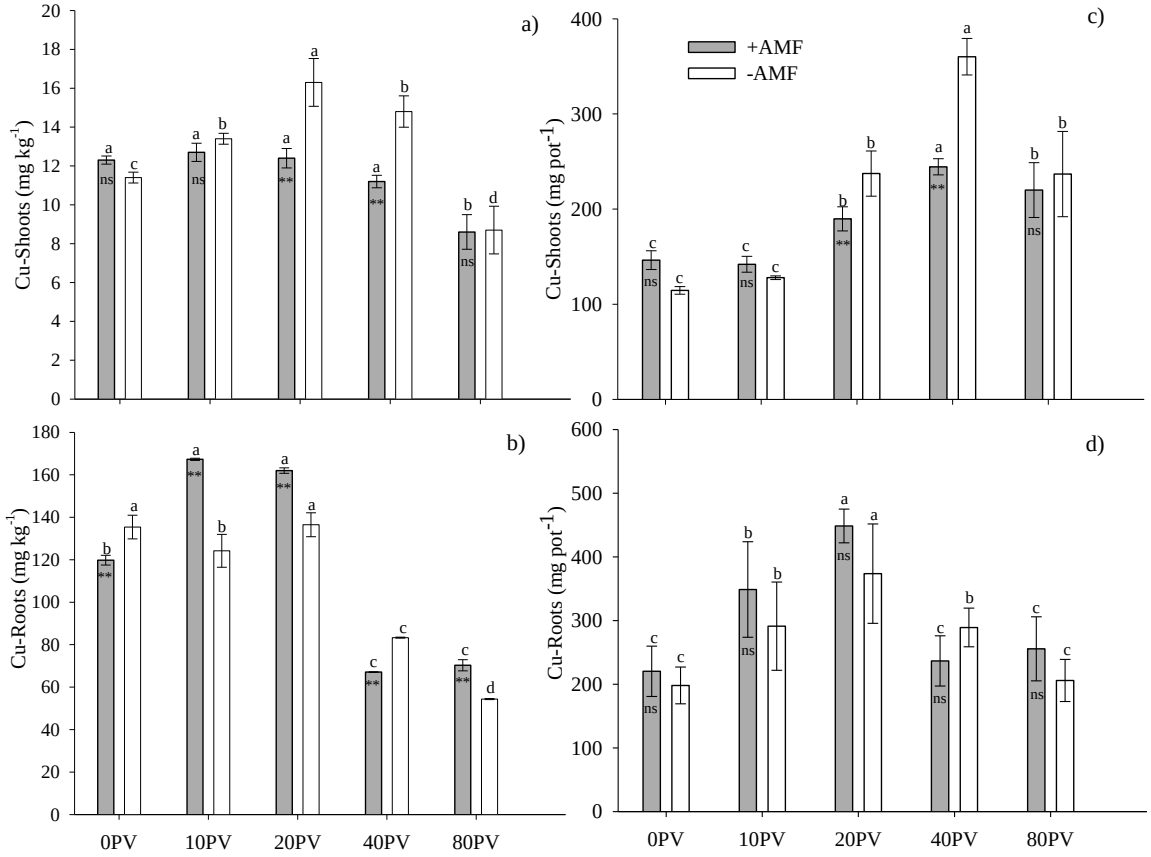

a)
b)
c)
d)
Cu-Shoots (mg kg-1)
Cu-Roots (mg kg-1)
Cu-Shoots (mg pot-1)
Cu-Roots (mg pot-1)
+AMF
-AMF
0PV
10PV
20PV
40PV
80PV

Fig. 4

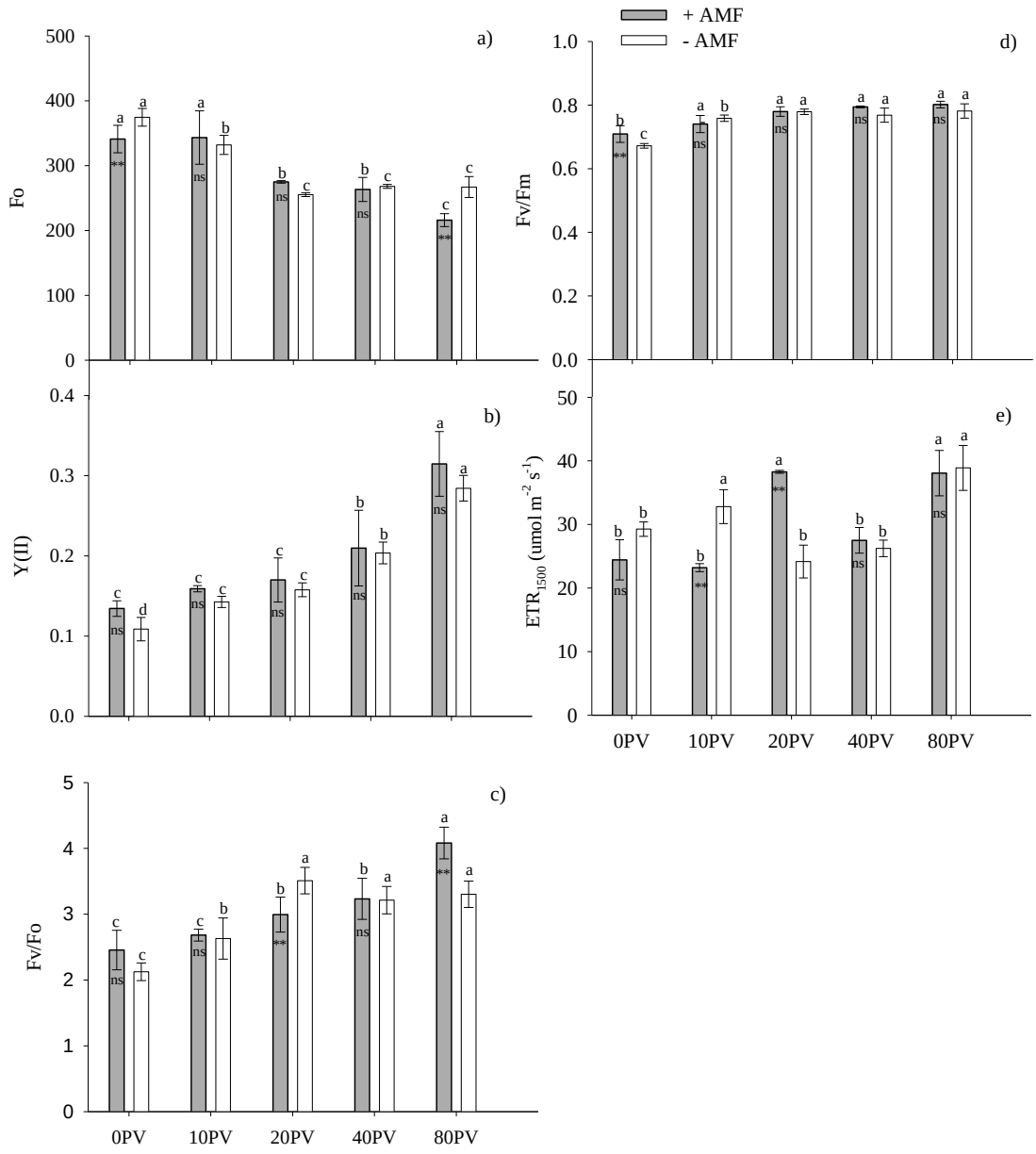

+ AMF
- AMF
a)
b)
c)
d)
e)
Fo
Y(II)
Fv/Fo
Fv/Fm
ETR1500 (umol m-2 s-1)
0PV
10PV
20PV
40PV
80PV

Fig. 5

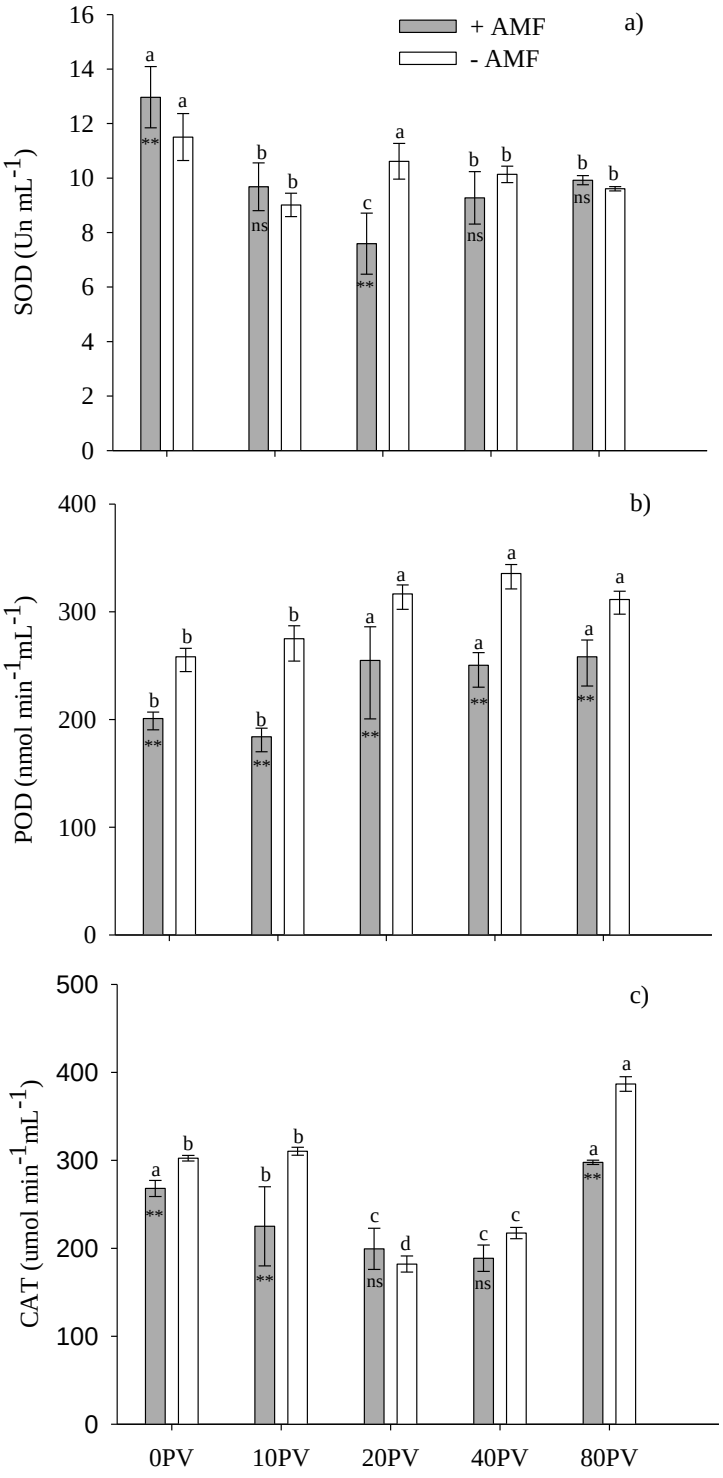
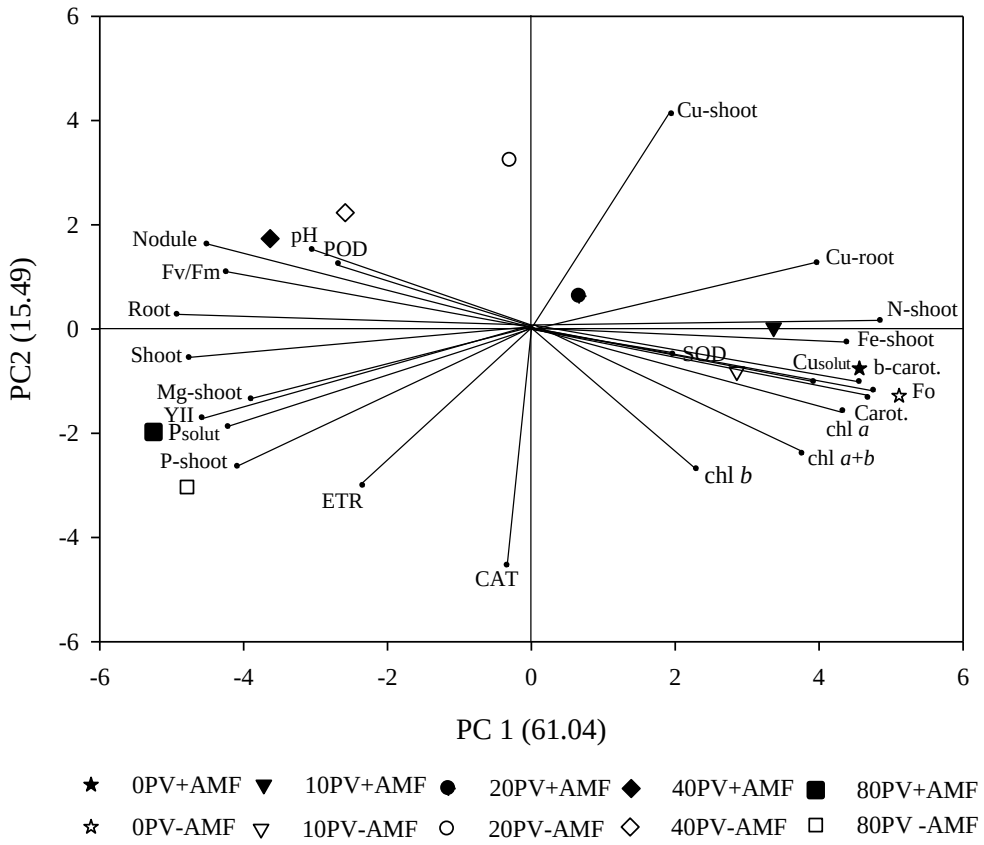

+ AMF
- AMF
a)
b)
c)
SOD (Un mL-1)
POD (nmol min-1mL-1)
CAT (umol min-1mL-1)
0PV
10PV
20PV
40PV
80PV

Fig. 6


6
4
2
0
-2
-4
-6
-6
-4
-2
0
2
4
6
PC2 (15.49)
PC 1 (61.04)
Cu-shoot
Nodule
pH
POD
Fv/Fm
Root
Shoot
Mg-shoot
YII
Psolut
P-shoot
ETR
CAT
Cu-root
N-shoot
Fe-shoot
SOD
Cusolut
b-carot.
Fo
Carot.
chl a
chl a+b
chl b
0PV+AMF
10PV+AMF
20PV+AMF
40PV+AMF
80PV+AMF
0PV-AMF
10PV-AMF
20PV-AMF
40PV-AMF
80PV -AMF

3 CONSIDERAÇÕES FINAIS

Os resultados deste estudo demonstram que é possível utilizar o bagaço da uva em processos de vermicompostagem, para obtenção de um adubo orgânico com características aceitáveis pela legislação brasileira para o uso agrícola. A adição deste vermicomposto ao solo reduz o teor de Cu disponível no solo, eleva os teores de nutrientes nos tecidos da planta, reduz os efeitos tóxicos do Cu nas células e favorece o crescimento de *C. ensiformis*. A inoculação por fungo arbuscular favorece a absorção de nutrientes em *C. ensiformis* cultivada em solos com baixa fertilidade. A fitoextração do Cu por *C. ensiformis* é aumentada com a adição ao solo arenoso de doses intermediárias do vermicomposto. O fungo arbuscular reduz o teor de Cu na parte aérea das plantas em solo contaminado por este metal. A adição de vermicomposto à base de bagaço de uva ao solo contaminado por Cu associado à inoculação com fungo micorrízico arbucular em *C. ensiformis* possui potencial para fitoestablização.

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