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ORIGINAL ARTICLE

Arbuscular mycorrhizal fungi activity in Haplic Luvisol on the semi-arid region of Pernambuco – Brazil

Atividade de fungos micorrízicos arbusculares em Luvissolo Háplico do semiárido Pernambucano – Brasil

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ABSTRACT: The use of microorganisms aiming to improve the availability of nutrients for plants is an important and necessary practice for agriculture. Thus, studies on the AMF occurrence in the brazilian northeastern and efficiency tests using native plants in the region have been intensified on recent decades. Aiming to determine the most probable number (MPN) of infective propagules in the area with native vegetation on the semi-arid region of Pernambuco, municipality of Sertânia, quantify the soil protein related to easily extractable glomalin (SPREEG) and to total glomalin (SPRTG), experiments were conducted in a greenhouse. For this study 10 composite soil samples were collected, with points randomly defined. Samples were homogenized and analyzed for physical and chemical characteristics. Being used for immediate examination and preparation trap cultures (one three-month cycle of propagation) to AMF, using as host plants sorghum (*Sorghum bicolor* L. Moench), and peanut (*Arachis hypogaea* L.). To determine the MPN of infective propagules of AMF in Haplic Luvisols, soil inoculum samples were diluted in the proportions of 0, 1:10, 1:100 and 1:1000 (soil inoculum: diluent soil, v:v) with 5 repetitions each, using maize (*Zea mays* L.) as host plant. The direct count (DC) differed significantly from the indirect (IC) of AMF with averages of 961.3 and 517.4 glomerospores per 100g soil, respectively. The NMP of infective propagules of AMF propagules found was 23 cm^{-3} and SPREEG and SPRTG were approximately 0.46 and 0.26 mg glomalin g^{-1} aggregate, respectively.

RESUMO: *O uso de micro-organismos com o objetivo de melhorar a disponibilidade de nutrientes para as plantas é uma prática importante e necessária para a agricultura. Assim, trabalhos sobre a atividade de fungos micorrízicos arbusculares (FMA) no Nordeste do Brasil e testes de eficiência utilizando plantas nativas da região vêm se intensificando nas últimas décadas. Objetivando determinar o número mais provável (NMP) de propágulos infectivos em área com vegetação nativa do semiárido pernambucano, no município de Sertânia, e quantificar o teor de proteínas do solo relacionadas à glomalina total (PSRGT) e facilmente extraível (PSRGFE), foram conduzidos experimentos em casa de vegetação. Foram coletadas 10 amostras compostas de solo, sendo os pontos definidos aleatoriamente. As amostras foram homogeneizadas e analisadas quanto às características físicas e químicas. Sendo utilizadas para exame imediato e preparo de culturas-armadilha (um ciclo de multiplicação de três meses) para FMA, empregando-se como plantas hospedeiras sorgo granífero (Sorghum bicolor L. Moench) e amendoim (Arachis hypogaea L.). Para a determinação do NMP de propágulos infectivos de FMA no Luvissolo Háplico, amostras do solo-inóculo foram diluídas nas proporções de 0, 1:10, 1:100 e 1:1000 (solo-inóculo: solo diluente, v:v), com 5 repetições cada, e tendo o milho (Zea mays L.) como planta hospedeira. Na contagem direta (CD) e indireta (CI) dos FMA, foram encontrados valores 961,3 e 517,4 glomerosporos 100g $solo^{-1}$ respectivamente. O NMP de propágulos infectivos de FMA encontrado foi de 23 propágulos cm^{-3}, e as proteínas do solo relacionadas à PSRGFE e à PSRGT ficaram em torno de 0,46 e 0,26 mg glomalina·g^{-1} agregado, respectivamente.*

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1 Introduction

The edaphic microbiota is an essential component of the soil-plant system (Gomide et al., 2009). Of soil microorganisms, the most intimately and necessarily associated with roots are the arbuscular mycorrhizal fungi (AMF) (Berbara et al., 2006), belonging to the phylum Glomeromycota (Oehl et al., 2011).

Their hyphae act as an extension of the root system, absorbing a much larger quantity of nutrients than that achieved by non-colonized roots (Miranda, 2008), also increasing the water absorption by the plant (Linderman, 2000; Smith & Read, 2008). The main advantage of these hyphae is related to the fact of absorbing with greater ease nutrients with low mobility in soil, such as P, Zn and Cu (Miranda & Harris, 1994; Smith & Read, 2008; Stümer & Siqueira, 2013; Priyadharsini & Muthukumar, 2015). The mycelium of AMF also adds soil particles (Rillig & Mummey, 2006) and acts in the soil carbon stock through the production of glomalin, a glycoprotein (Mergulhão et al., 2008). Viscous substance, with adhesive capacity (Mergulhão et al., 2011).

The population of AMF in the soil, through the multiplicity of their species, is considered one of the most important factors for maintaining biodiversity and ecosystem functionality (van der Heidjen et al., 1998; Moreira et al., 2007).

In a native caatinga area, in Alagoas, the most probable number (MPN) of infective propagules of AMF ranged from 0.82 to 13.18 g^{-1} soil (Souza et al., 2003), while in a native area, used for disposal of gypsum mining waste, in Pernambuco, the MPN of propagules was significantly lower in the impacted area (17g^{-1} of soil) than in the preserved one (540 g^{-1} soil) (Mergulhão et al., 2007).

Sousa (2013), evaluating the relationship between the diversity of land use systems and AMF communities in the semi-arid region of Paraíba, concluded that the presence of trees favored the sporulation, the mycorrhizal colonization and the number of infective propagules of AMF in the land use systems. Gattai et al. (2011), working with lead-contaminated soils in the semi-arid region of northeastern Brazil, found that regardless of the season, either dry or rainy, the MPN of infective propagules was drastically reduced. Yet Mergulhão et al. (2007) observed a higher MPN of infective propagules of AMF during the dry season.

The use of microorganisms aiming to improve the availability of nutrients for plants is an important and necessary practice for agriculture (Freitas, 2007; Priyadharsini & Muthukumar, 2015), since the presence of these beneficial microorganisms in the rhizospheric region and their activities make the dynamic resources available to plants, besides preserving soil fertility (Priyadharsini & Muthukumar, 2015). As seen by Ortiz et al. (2015), microbial activities, regardless of the microbial origin, may function in the plant as an adaptive response to limited water availability. Thus, the aim of the study was to check the AMF activity from the assessment of the most probable number of infective propagules and the glomerospore density in an area with native vegetation in the semi-arid region of Pernambuco, in the municipality of Sertânia, and to quantify the content of soil proteins related to glomalin.

2 Materials and Methods

The soil used was Haplic Luvisol, previously called Bruno non-calcic, from the Experimental Station of the Agronomic Institute of Pernambuco, in Sertânia - PE, 08º 04' 25" S and 37º 15' 52" W, at an altitude of 558 m. The climate of the municipality of Sertânia, according to Köppen classification, is hot semi-arid. The annual rainfall rate in the municipality is 635 mm, with seven months of drought, the highest annual rainfall values occurring in the months of March and April, and annual average temperature of 25 ºC (Mascarenhas et al., 2005).

For this study 10 composite soil samples were collected, with points randomly defined. The collected soil was sieved (2.0 mm diameter sieve) and homogenized. Part of the samples was designed to direct count (DC) and another was intended for experiments and analysis of chemical and physical characteristics (Table 1) according to the methodology recommended by EMBRAPA (2009). The experiments I and II were conducted in greenhouse.

The composite samples were used for direct (DC) and indirect count (IC) of glomerospores, from the preparation of trap cultures (Sieverding, 1991), using grain sorghum (*Sorghum bicolor* (L.) Moench) and peanut (*Arachis hypogaea* L.) as host plants (experiment I). The samples were placed in pots (ten in total) and kept in a greenhouse during a multiplication cycle (three months). Each plastic pot (with capacity of 3 kg soil pot^{-1}) was filled with 1 kg of soil relative to a point (of the 10 points) and 1 kg of autoclaved washed sand (diluent), thus obtaining the ratio of 1:1.

Table 1. Chemical and physical characteristics of the Haplic Luvisol originating from an area with native vegetation in the municipality of Sertânia, PE.
Tabela 1. Características químicas e físicas originário de área com vegetação nativa no município de Sertânia, PE.

Chemical characteristics									
Area	P	Ca	Mg	K	Na	Al	H	CTC	pH
	mg dm^{-3}	Cmolc dm^{-3}							H_2O 1:2.5
Sertânia – PE	12	3.9	1.95	0.32	0.18	0.0	3.3	9.6	6.0

Physical characteristics							
	Granulometry (%)				Residual moisture	bd*	pd**
Area	Coarse sand	Thin sand	Lime	Clay	%	g cm^{-3}	
Sertânia – PE	50	19	21	10	2	1.6	2.65

*Bulk density (bd); **Particle density (pd).

In the peanut seeds, due to contamination with fungi, disinfestation was performed by immersion in 70% alcohol for 30 seconds, followed by 1 minute in 0.2% sodium hypochlorite, being subsequently rinsed with distilled water and autoclaved (sterilized), seven times (Vincent, 1970 modified by Silva, 2012). Sorghum was sown directly in the pots, each pot received 100 seeds at the moment in which the peanut seedlings were transplanted, two seedlings per pot. Thus, each pot received two peanut seedlings and 100 sorghum seeds.

The trap culture did not receive any type of fertilizer or nutrient solution, having the soil itself as a source of nutrients for plants (Table 1). Moisture maintenance was performed from irrigation with distilled water, according to the need of plants. The same was extended for a period of three months and at the end of this period the pots went through a dry period, when irrigation was stopped to facilitate the germination and the dormancy break of the glomerospores, making thereby the 1st cycle of these crops. After the water stress period, samples were collected from these pots (100 g soil pot^{-1}). Spores of AMF were extracted from the soil by wet screening (Gerdemann & Nicolson, 1963), followed by centrifugation in water and 50% sucrose (Jenkins, 1964). This material went to channeled plates to perform the count of glomerospores with the aid of a stereomicroscope, performing the indirect count (IC) of AMF spores of the 1st cycle.

For the assessment of the MPN of infective propagules of AMF in the Haplic Luvisol (experiment II), it was used the technique described by Feldmann & Idczak (1992). For this study 20 pots were mounted (diposable cups of 250 mL each), with samples composed of field soil (soil inoculum) and diluent soil (sand) in a serial dilution system: 0, 1:10, 1:100 and 1:1000, with 5 replications each, having maize (*Zea mays* L.) as host plant (two seedlings pot^{-1}). The diluent soil (sand) was autoclaved at 121 °C for 1 hour, for two consecutive days, and then air dried. Each pot received 200 g of substrate (soil + sand or mixture + sand, according to their respective dilutions).

After the first 10 days of the experiment, thinning was carried out, leaving one plant per pot, and 20 mL of the nutrient solution of Hoagland and Arnon modified (Jarstfer & Sylvia, 1992) were applied, free of P. The nutrient solution was applied weekly to the end of the experiment. Water maintenance was performed from the irrigation with distilled water. These pots were in the greenhouse for a period of 30 days.

At the end of the period, the plants were harvested and the roots separated, washed, diaphanized with KOH (10%) and stained with Trypan blue (0.05%) in lactoglycerol (Phillips & Hayman, 1970). To determine the MPN, it were assigned the signs (+) for presence and (-) for absence of typical structures of AMF in the roots, observed in the stereomicroscope, being estimated by Cochran table (1950) and with results expressed in numbers of propagules by cm^{-3} substrate. When necessary, the microscope was used to make sure of the mycorrhizal colonization.

Analyses were performed at the Mycorrhizae Laboratory of the Federal University of Pernambuco. Soil proteins related to easily extractable (SPREEG) and total glomalin (SPRTG) were extracted from the soil following the methology of Wright & Upadhyaya (1998). For SPREEG, it were added three subsamples of 0.25 g aggregate (grade 1-2 mm), 2 mL sodium citrate (20 mM; pH 7.0), autoclaved at 121 °C for 30 minutes and with subsequent centrifugation at 10,000 g for 5 minutes. The SPRTG was extracted from the addition of 2 mL sodium citrate (50 mM; pH 8.0) to the sediment resulting from the SPREEG extraction, being conducted cycles (1h at 121 °C) of extraction in an autoclave and centrifugation at 10,000 g for 5 minutes. Autoclaving cycles of 1 hour were done until the extract lost the tile color, or rather reddish-brown color, characteristic of the presence of glomalin. The SPRG present in the supernatant were quantified by the Bradford (1976) method, having as standard curve the bovine serum albumin (BSA).

The data obtained in the direct count (DC) and in the indirect count (IC) were analyzed by the software ASSISTAT 7.7 Beta, being held ANOVA and test for means comparison based on the Tukey test ($p < 0.05$).

3 Results and Discussion

The number of glomerospores of the Sertânia soil (Haplic Luvisol) shown in the direct count (DC) differed significantly from that quantified in the indirectly count (IC), i.e., in the multiplication (trap culture), with averages of 961.3 and 517.4 per 100 g soil, respectively. As the DC samples were collected near the roots of the plants in the study area, it must have influenced this greater value in relation to the IC, since the rhizosphere is where the largest amount of microorganisms is found. Another factor may have been the own adaptation of microorganisms to a new situation, pots in the greenhouse, a habitat completely different of theirs in the soil as a whole. This should probably have been a factor to inhibit the sporulation of certain species.

When compared to the direct count of this study, Nobre et al. (2010), in soils under alley cropping systems with legumes in Maranhão, obtained in their results a greater glomerospore density (200 glomerospores 100 g $soil^{-1}$) in the rhizosphere of leucaena and sombreiro. Silva et al. (2007) found that the number of glomerospores, in soils with cultivation of 'sabiá' or leucena in municipalities in the state of Pernambuco, ranged from 69 to 437 glomerospores per 50 g of soil, respectively, in Serra Talhada, with 'sabiá' cultivation, and in Caruaru, with leucaena. Souza et al. (2003), working in a caating area, , observed variation between 34-860 spores per 100 g soil, in a study developed in permanent plots in the Reference Center for Recovery of Degraded Areas of Caatinga (CRAD), in Petrolina, noted that the glomerospore density in soil samples varied between 30 and 300 glomerospores (50 g $soil^{-1}$) and that the plots CaatD and CaatC, with the highest and the lowest degree of vegetation degradation, had lower values of glomerospores (40-53 glomerospores 50 g $soil^{-1}$).

While Dantas et al. (2015), evaluating the occurrence of AMF in the rhizosphere of fruits in development, under organic management, compared to a native vegetation area in the semi-arid region of Ceará, detected high density of AMF glomerospores (in 100 mL soil) in the natural vegetation area, but not differing statistically from the other areas.

On the other hand, Mello et al. (2012) found in their studies, in a preserved caatinga area in Pernambuco, a very low number of glomerospores (1 spore·g^{-1} soil), when compared to the

present work. A fact that may have been given caused by the high content of organic matter located in the area, which may have benefited the development of organisms that prey on glomerospores (Mello et al., 2012). Or still, as seen in Souza et al. (2003), in soils with high levels of P, there is a reduction in their production. Santos et al. (2014) investigated the influence of seasonality on the average number of AMF glomerospores (average density = 9.9 ± 0.6 glomerospores g^{-1} soil) in the liana forest fragment studied (Vitória da Conquista-BA), justifying that the AMF, too, are influenced by various environmental factors.

Oliveira et al. (2009), studying the soil of a dune mining area with reforested restinga in the Paraíba coast, quantified 50 glomerospores 50 g $soil^{-1}$, a figure considered low when compared to the present study, or compared to other works in native area as previously mentioned. Silva et al. (2001) found, in areas of native caatinga and degraded by mining, a glomerospore level always below 160 glomerospores 100 g $soil^{-1}$. According to these data, it is possible to suggest that the work in areas with soil with preserved native vegetation, which have their micro-, meso- and macrofauna intact, have higher values of glomerospores than those in areas which had their soils degraded, such as mining soils, which showed much lower values. These figures only show how a soil can be harmed with its reckless use, and that it may turn out to be depleted with indiscriminate use.

In contrast, Teixeira-Rios et al. (2013), evaluating a caatinga area degraded by limestone mining in Campo Formoso, noted that the number of glomerospores showed no significant difference between preserved areas (164.0 ± 44.0 glomerospores 100 g $soil^{-1}$) and degraded areas (186.0 ± 47.0 glomerospores 100 g $soil^{-1}$). According to Entry et al. (2002), a lower number of glomerospores can also be related to high pH and low nutrient availability in the soil.

The MPN of infective propagules of AMF found in this study (23 propagules cm^{-3}) was intermediate to the values found by Silva et al. (2001), which ranged from 0.00 to 35.92 in the dry season and from 1.10 to 27.22 in the rainy season. According to these authors, the number of glomerospores in the soil has always been higher than the number of infective propagules, except for the soil impacted by the action of copper mining (tailings basin) during the rainy season. This may have occurred because the spores are more resistant structures than other types of propagules, as hyphae and vesicles, and can remain in the soil for longer (Silva et al., 2001). It is also possible that unviable spores have been counted; additionally, some species of AMF present dormancy and, in that case, they are not detected by the MPN technique (Silva et al., 2001). This may have occurred in this study, with the presence of more resistant structures like spores, or these being in a state of dormancy or unviable.

The MPN found by Sousa et al. (2013) was high compared to that found in this study, it ranged from 39 propagules cm^{-3}, having as land use system the palm without trees, to 540 propagules cm^{-3}, in the presence of buffel grass with trees. According to Ganesan & Veeralakshmi (2006), this value for the buffel grass may have been justified by the fact that it is considered a good host plant for the multiplication of *Glomus fasciculatum*, due to its rapid growth and abundant root system.

Gattai et al. (2011), working with lead-contaminated soils in the semi-arid region of northeastern Brazil, obtained, for the MPN of infective propagules, values of 140 in the dry season and 350 in the rainy season in uncontaminated soil, different from that observed in soil with excess lead (270 mg kg^{-1}), with values of 12 in the dry season and 40 in the rainy season.

Regarding soil proteins related to easily extractable glomalin (SPREEG) and soil proteins related to total glomalin (SPRTG), the values were approximately 0.46 and 0.26 mg glomalin g^{-1} aggregate (1-2 mm), respectively. In soils, glomalin is a relatively stable protein (Rillig et al., 2003; Wright & Upadhyaya, 1998); probably, the soil characteristics and climatic conditions can influence their concentrations in the soil, since the presence and the type of vegetation affect their production (Sousa et al., 2014; Bird et al., 2002). Moreover, Sousa et al. (2014), evaluating the occurrence of AMF in different successive stages of caating in the semi-arid region of Paraíba, found that regardless of the area, SPREEG and SPRTG did not differ significantly.

Sousa et al. (2011), working with Luvisol in the semi-arid region of Paraíba under savanna vegetation, used as pasture, noted that the highest glomalin levels (0.97 mg g $soil^{-1}$) are possibly explained by the pH of 6.08; since fungi tend to predominate in acid soils, as in alkaline soils there is more competition with bacteria and other organisms (Weil & Brady, 2016). Sousa et al. (2013) found that in the relationship between the diversity of land use systems and the AMF communities in the semi-arid region of Paraíba, the largest contents of SPRG were recorded in the palm production system, getting around 1.14 mg g $soil^{-1}$, in which plants also showed a higher percentage of mycorrhizal colonization, suggesting that large amounts of photosynthates were being allocated to the AMF by plants, what possibly stimulated the production of this protein.

Mergulhão et al. (2010), working with waste and gypsum mining soil in the semi-arid of Pernambuco, in the region of the Araripe, found values ranging from 0.01 mg g $soil^{-1}$, in the waste, to 0.9 mg g $soil^{-1}$, in the caatinga preserved for easily extractable protein, and values ranging from 2.8 mg g $soil^{-1}$, in the waste, to 4.3 mg g $soil^{-1}$, in the caatinga preserved for total protein content. Thus, the glycoprotein can be used as an indicator of edaphic quality, considering that it pointed differences between the impacted soils and the native soil, preserved (Mergulhão et al., 2010).

There are still few and scattered data on the contents of total protein and easily extractable protein in Brazilian research, and commonly they are complementary to other data, such as soil microbial C, soil organic C, aggregation, diversity, spores and infectivity of AMF (Mergulhão et al., 2010; Purin & Filho, 2010).

4 Conclusions

Studies on the occurrence and efficiency tests with AMF are needed to increase knowledge on the diversity and the potential of use of these fungi in the semi-arid region of Pernambuco,

and the soil samples of the Haplic Luvisol (Sertania soil) showed mycorrhizal activity with a significant number of glomerospores and infective propagules of AMF. The MPN of infective propagules of AMF found in the municipality of Sertânia (23 propagules cm^{-3}) is considered intermediate. The soil proteins related to easily extractable glomalin and total glomalin, corresponding to areas without abiotic stress, have values around 0.46 and 0.26 mg glomalin g^{-1} aggregate, respectively.

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