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Growth of mycorrhized seedlings of *Leucaena leucocephala* (Lam.) de Wit. in a copper contaminated soil

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Abstract

Due to the low infectivity potential of arbuscular mycorrhizal fungi (AMF) in a mining area located at the State of Bahia, Northeastern Brazil, the effect of mycorrhization on the seedlings of *Leucaena leucocephala* was investigated, in order to use this species for revegetation of the area. Caatinga soils from both, natural (control) and mining impacted areas, were used to maintain seedlings inoculated with *Glomus etunicatum*. The soil from the impacted area was diluted 0, 25, 50, 75 and 100% with soil from the control area. In general, the increase in the proportion of contaminated soil had a negative effect on plant growth. Inoculated plants presented greater height, leaf number and dry matter of roots and shoots than the non-inoculated plants, when cultivated in soil with up to 50% of contaminated soil. Higher spore number was produced in substrate with 25% soil contamination. Mycorrhizal colonization was higher than 40% in those treated with <50% disturbed soil. Proportions of copper contaminated soil higher than 50%, inhibited plant growth, development of the AMF in the roots and consequently, the benefit of mycorrhization. The pattern of Cu and P absorption in *Leucaena*, associated or not with *G. etunicatum*, is maintained when the soil is up to 50% contaminated.

Keywords: Arbuscular mycorrhizae; Heavy metals; Soil contamination; Soil disturbance; Copper

1. Introduction

The Brazilian Northeast region includes a semiarid area of approximately 9×10^5 km^2, half of it is still covered by the native caatinga vegetation that thrives predominantly on shallow, relatively fertile soils.

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The area has several mineral deposits that are mined in open pit operations, usually without reposition of the soil layer. Sites where the waste accumulates, remain for long periods with no or only very poor vegetation, if no effort is made to plant them. *Leucaena leucocephala* (Lam.) de Wit., commonly referred as leucaena, is one of the plants used in these revegetation actions.

The arbuscular mycorrhizal fungi (AMF) can alleviate plant toxicity produced by heavy metals, increasing plant tolerance (Leyval et al., 1997). Use of mycorrhizal inoculum tolerant to heavy metals has been indicated for growth of tree seedlings planted in contaminated tropical areas (Siqueira et al., 1999) allowing phytoremediation of heavy metal polluted soils (Scheloske et al., 2001). For this, environmental and plant growth conditions, the fungal strain and the type of metal should be considered (Azcón-Aguilar and Barea, 1997). The ecological and technological importance of these fungi is clear when considering the need for rehabilitation of diverse polluted areas with potentially toxic compounds.

The development of low cost, effective inoculation methods will help revegetation programmes in Northeast Brazil. Considering the need for recovering an area disturbed by copper mining activities, the effect of AMF inoculation on *L. leucocephala* plants growing in soils with increasing proportions of copper mining waste was investigated.

2. Materials and methods

Soil samples were collected from two areas of "Mineração Caraíba" (Jaguarari municipality, Bahia State),from where copper is extracted. The first one had been used as a deposit of mining waste, consisting of the powder of the rock from which copper was extracted, and is called waste area. The second area corresponds to a preserved caatinga area and was taken as a control. From each area, 10 samples were collected at random from 5 to 20 cm layer. Analysis of composite subsamples presented the following characteristics: waste = 70, 484, 673, 3.6 and 53 mg dm^{-3} of soil of extractable P, Cu, Fe, Zn and Mn, respectively (Mehlich-1); pH 7.4; 5.7 g dm^{-3} of organic matter; granulometry: 93, 3 and 4% of sand, silt and clay, respectively; caatinga = 10, 0.4, 216, 4.6 and 3.5 mg dm^{-3} of soil of P, Cu, Fe, Zn and Mn, respectively; pH 6.1; 27.3 g dm^{-3} of organic matter; granulometry: 62, 24 and 14% of sand, silt and clay, respectively.

Soil from the two areas was mixed to provide an increasing proportion of soil from the waste area. The preserved caatinga soil (base of dilutions) was disinfested with Bromex (98% methyl bromide + 2% chloropicrine), a month before being used.

Ten days old seedlings of *L. leucocephala*, planted in plastics bags containing 2 kg of substrate, were inoculated with 100 spores of *Glomus etunicatum* (UFPE 06) obtained from the AMF culture collection of the Departamento de Micologia/UFPE. A filtrate of the soil inoculum, without AMF propagules, was added in the treatments without AMF to maintain the soil microbiota. The temperature and relative air humidity in the greenhouse were 23–32 °C and 50–81%, respectively. Ninety days after inoculation, plant height, leaf number, total dry matter, colonization and AMF spore density were evaluated. Plant material was dried at 65 °C; fresh roots (0.5 g) of each sample were stained (Koske and Gemma, 1989), and colonization evaluated by the transect method. Spore density was evaluated after wet sieving and sucrose centrifugation. Plant material was digested with a nitric-perchloric procedure and P, Cu, Fe, Zn and Mn concentrations were determined by colorimetry (P) and atomic absorption spectrophotometry (Cu, Fe, Zn and Mn).

The experiment was set up as a complete randomized design, in a 5 × 2 factorial arrangement, consisting of the five waste soil dilutions (0%-preserved caatinga soil; 25, 50, 75 and 100% of contaminated soil) and the two inoculation treatments (*G. etunicatum* and non-inoculated control), with five replicates. Data were submitted for analyses of variance and averages compared by the Student L.S.D. test, at 5% probability. Prior to analysis, root colonization and spore density data were transformed into arcsine $\sqrt{x}/100$ and $\log x + 1$, respectively.

3. Results

Inoculation with *G. etunicatum*, favoured seedling growth in the disinfested, preserved caatinga soil, measured either as dry matter or as plant height and leaf number, being 14, 4 and 4 times higher than those of the non-inoculated treatment (Table 1). In this late