

Tolerance of *Anadenanthera peregrina* to *Eucalyptus camaldulensis* and *Eucalyptus grandis* Essential Oil as Condition for Mixed Plantation

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ABSTRACT

With the purpose of selecting the species of woody Caatinga for mixed plantations with *Eucalyptus* spp., the allelopathic effects of *E. camaldulensis* and *E. grandis* essential oil were studied on the growth activities of *Anadenanthera peregrina*. The plants were closed in glass chambers in the presence of volatile oil of *E. camaldulensis* or *E. grandis* at the concentration of 13 nl.cm⁻³. The number of leaves, height and diameter at soil level were compared before, immediately after and after 30 days. Chlorophyll a and b, carotenoids and dry mass were evaluated after the treatment application. There was no inhibitory effect of *E. camaldulensis* and *E. grandis* oils on *A. peregrina*. *E. camaldulensis*, which was more adapted to semi-arid conditions, was planted in mixture stands with two native legume species, inoculated with *Rhizobium* and arbuscular mycorrhizal fungi. *E. camaldulensis* did not inhibit native species growth after two years of cultivation.

Key words: Allelopathic, Caatinga; angico

INTRODUCTION

The negative direct or indirect plant effects over the biotic environment such as other plants or soil microorganisms are known as allelopathy. Plants release secondary metabolites into the environment, air, water and soil, through volatilization and root exudates (Deuber 1992). The *Eucalyptus* and *Corymbia* species, considered as notorious allelopathic tree although most often study plants, are commonly used in afforestation models over the world, due to their fast growth, good adaptation to different soil and climatic conditions and high timber value. Several studies

have revealed that some *Eucalyptus* and *Corymbia* species may exert allelopathic effect by soluble metabolites lixiviation (May and Ash 1990; Kohli 1990) or by volatiles terpenoid compounds (Kohli and Singh 1991). The soluble fraction is transported by percolation and is absorbed by others plants, which can be used by the microorganisms or adsorbed by the clays, participating in soil cation exchange capacity (CEC). The effects exerted by the allelopathic terpenoid volatile compounds that are found in the environment in the form of vapor (Muller et al.1964) depend on the partial pressure of terpenoids in the air (Singh et al. 1993). The

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bactericide effect exhaled by the leaves of *Eucalyptus globulus* (volatile fraction) has been reported for the medical purposes which allows the characterization for the purpose as stated in the medical world pharmacopedy.

It is also known that *Eucalyptus* spp. produces inhibitory substances for the soil bacterial growth (Della-Bruna et al. 1989; Moura et al. 1996), but, on the contrary, fungi population can be favoured (Della-Bruna et al. 1989; Moura et al. 1996).

Monocultures of some *Eucalyptus* species, over 40 years can retard the succession causing understory suppression (Del Moral et al. 1978; Bhaskar and Dasappa 1986; Singh et al. 1993). *Eucalyptus* can decrease the diversity in understory vegetation and borders of plantation in comparison to adjacent preserved areas (Suresh and Rai 1987; Kohli et al. 1992). Kohli et al. (1998) reported that *E. globulus* and *C. citriodora* oil inhibit the growth of the weed *Parthenicum hysterophorus*, through the inhibition of germination and cellular respiration, reduction of chlorophyll content and increment increase of water loss, leading to complete plant wilt after 15 days of oil exposition.

In Brazil, one of the most important programs of irrigation is on the São Francisco river. Known as Jaíba Project, it was established to increase the agricultural production and economic and social growth of the region. The Jaíba reserve is one of the largest protected areas of woody Caatinga and its natural vegetation is classified as "Dry Deciduous Forest" (Rizzini 1979), which has been subject to the destructive effects of deforestation and fire. Such environmental impact may result not only in the destruction of the biodiversity (flora and fauna), the chemical, physical and biological loss of soils, but also a perilously rapid loss of the wood stocks favoured by wood extraction, creating an impact on the local populations. It is important to consider that the affected populations are characterized by a high vulnerability, since they are among the poorest in the region. The demand for the wood by the farmers has become a continuous threat to the preserved area, revealing a need for a project of wood and energy provision for the local populations. Mixed-species plantations represent an alternative for revegetation with fast growing native species. Similar works in other regions such as Hawaii (De-Bell et al. 1985), Asia (Khanna 1997), Australia (May and Attiwill 2003), and Brazil (Gonçalves 2000) showed the advantages of legume use for increasing the plant growth and

improving the soil fertility. For example, in a *Eucalyptus* plantation in Brazil showed the benefits of inoculated leguminous species for the reforestation of a riparian forest (Marques et al. 2001).

Anadenanthera peregrina ("angico-vermelho") is representative of Brazil's Caatinga biome and is a semideciduous plant, attaining up to 30m height and 90cm of diameter at breast height (DBH). In "Cerrado" and "Caatinga" biomes, this species is shorter (3 a 15 m) (Carvalho 1994). *A. peregrina* (angico) plants has been reported for the reforestation of degraded areas and for mixed plantings, mainly due to their fast initial development (Lorenzi 1992).

The aim of this work was to examine the tolerance of *A. peregrina* to *Eucalyptus* essential oil and to test this effect under the field conditions. The plant growth under the mixed and pure stands was compared.

MATERIALS AND METHODS

Effect of *E. camaldulensis* and *E. grandis* essential oils

The samples of adult leaves that had just fallen from 10 randomly selected trees of each species from *E. camaldulensis* and *E. grandis* were collected and sealed in plastic bags and stored at 4°C until processed (within two weeks). About 1.0 Kg of leaves was taken for essential oil extraction, obtained by steam-distilled according to Brazilian Pharmacopoeia 4^a ed. The gravimetric method (Brazilian Pharmacopoeia 4^a ed.) was used to determine the water content and volatile compounds in the leaves samples after drying at 100°C ± 5°C to constant weight.

The seeds of *Anadenanthera peregrina* (L.) Spegazzini were collected in the Legal Reserve of Jaíba-Minas Gerais. After physical scarification and water immersion for 24 h, the seeds were germinated in a humid chamber at 29°C. Seedlings about 10 cm high were transplanted into plastic pots of 200 g, filled with soil collected from the Jaíba Project. The fertilizers used included 32 g of N, 40 g of P₂O₅, and 16 g of K₂O per m³/subsoil. Six-week-old plants of angico placed in the chambers (7500 cm³) were fumigated with 13.0 nl cm⁻³ of *E. camaldulensis* and *E. grandis* oil vapours, separately. The chambers of each native species were fumigated with the oil of *E. camaldulensis* and *E. grandis*, respectively as

follow: 1 - oils maintaining 100µl; at 48 h intervals the old vapours in the chambers were replaced by fresh ones. 2 - A chamber without oil vapours served as control. 3 - Plants which were maintained outside the chamber were considered as a check. Six replicates were made for each treatment/species using a randomized design. Before oil exposure and 5 and 35 days after the treatment, the number of leaves and height and diameter of each shoot were determined. After 35 days of exposure, the fresh and dry biomasses were determined. The *A. peregrina* blades leaves were divided into "leaflets" (pinnately), presenting also the rachis divided into many petioles and petiolules and because of this, it was difficult to measure the chlorophyll fluorescence in photosynthesis analyser. Alternatively, the plants of *A. peregrina* had the chlorophyll a, b and carotenoids content estimated according to Linchenthaler and Wellburn (1983), while the calculations were done as suggested by Daizy and Kohli (1991).

Effect of mixed plantation on *A. peregrina* growth

The experimental site (1.5 ha/site) was cleared of Carrasco (Araujo 1998) plants and cultivated as follows: *E. camaldulensis* intercropped with the native species *A. peregrina* and *Myracrodruon urundeuva*. The experimental design randomized block with nine treatments and three replicates blocks was followed using a density 3 x 2 m in areas of Jaíba Project, which had lost original vegetation. Excluding the buffer trees, there were 40 plants per plot at the monoculture (plot size was 18 x 20 m), and in the mixed-species plots 60 trees occupied 540 m². These nine treatments were irrigated for about 10 months.

The plots were cultivated as follows: a single plantation of *A. peregrina*; a single plantation of *A. peregrina* inoculated with *Rhizobia* and spores of arbuscular mycorrhizal fungi (AMF), a single plantation of *E. camaldulensis*, a single plantation of *E. camaldulensis* + AMF, a single plantation of *M. urundeuva*, a mixed plantation of *A. peregrina* + *E. camaldulensis* + *M. urundeuva*, a mixed plantation of *A. peregrina* (*Rhizobia* + AMF) + *E. camaldulensis* + *M. urundeuva*, a mixed plantation of *A. peregrina* + *E. camaldulensis* (AMF) + *M. urundeuva*, a mixed plantation of *A. peregrina*

(*Rhizobia* + AMF) + *E. camaldulensis* (AMF) + *M. urundeuva*.

Inoculants

The slow growing rhizobia strain BHICB-A10 was previously isolated from the nodules of *A. peregrina* at the Jaíba Project forest reserve, and screened for their effectiveness, under greenhouse and nursery conditions. The bacterial inocula were provided at 1.0ml per pot (10⁷ cfu/ml), according to Somasegaran and Hoben (1985). Mycorrhizal fungi used were *Gigaspora margarita*, *Scutelospora heterogama* and *Glomus etunicatum* from UFMG laboratory collection. Endomycorrhizal inoculation was accomplished by placing 1.0ml of suspension composed by 50 spores per species and 150 spores/ml in total of three species (*G. margarita*, *S. heterogama* and *G. etunicatum*) into each pot.

Sampling and analyses

Growth parameters, including the diameter at 30 cm above the ground and the height of all the plants, were recorded after 0.5, 10, 15 and 20 months of the growth. The data were statistically analyzed by ANOVA and the means were compared by the Tukey's. The means were compared for the growth in the field only at 20 months.

RESULTS AND DISCUSSION

Evaluation of *E. camaldulensis* and *E. grandis* oils effect on *A. peregrina* plants in the laboratory

The results showed that leaves production, height growth (Fig. 1 A, B and C, D.), and fresh and dry biomass production of *A. peregrina* plants (Fig. 2 A, B and C, D) were not affected by the essential oils of *E. camaldulensis* and *E. grandis* after 35 days of exposure. The effect on the number of leaves and biomass production was influenced by the incubation procedure, especially in shoot parts. The chlorophyll a, b and carotenoids analysis (Fig. 3 A and B) showed that *A. peregrina* plants were not susceptible to the inhibitory effect of essential oils of *E. camaldulensis* and *E. grandis*. *A. peregrina* plants showed high tolerance to the inhibitory effects of both *E. camaldulensis* and *E. grandis* at 13 nl. cm⁻³.

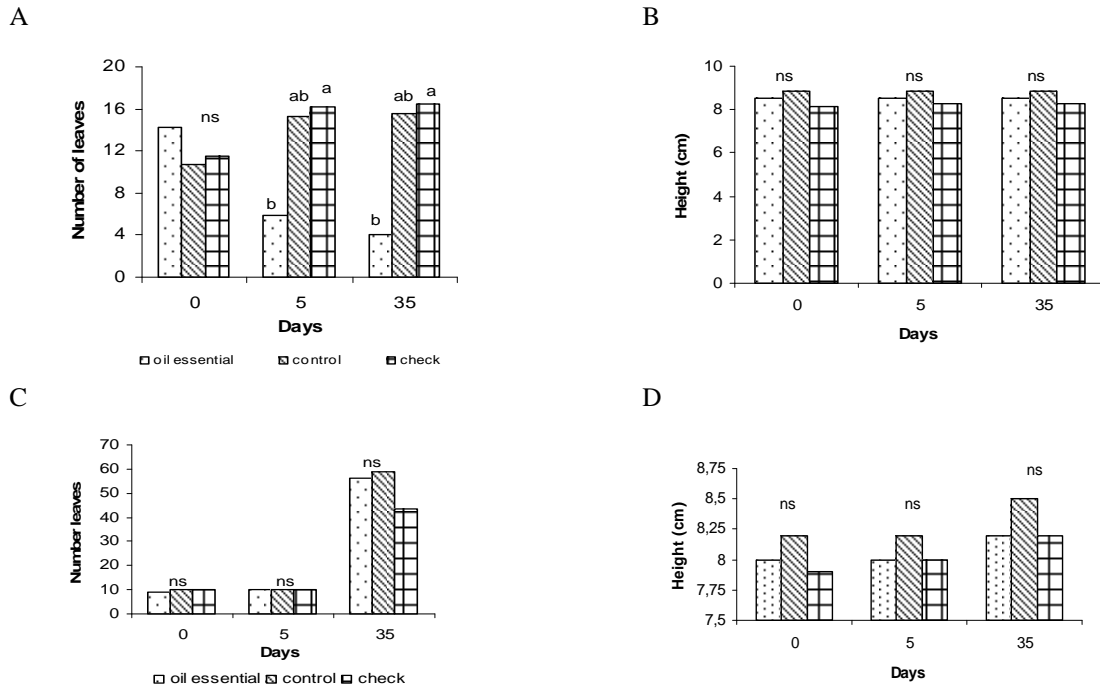


Figure 1 - *E. camaldulensis* oil effect in *A. pegrina* leaves number before and after 5 and 35 days of oil exposition (A), and plant height (cm) before and after 5 and 35 days of oil exposition (B). *E. grandis* oil effect in *A. pegrina* leaves number before and after 35 days of oil exposition (C), and plant height (cm) before and after 5 and 35 days of oil exposition (D). Control= plants incubated without oil; oil= plants incubated with oil, and check = plants not incubated). Means with different letters are significantly different as determined by Tuckey multiple-range test at the 5% confidence level ($P \leq 0.05$). Not significantly different.

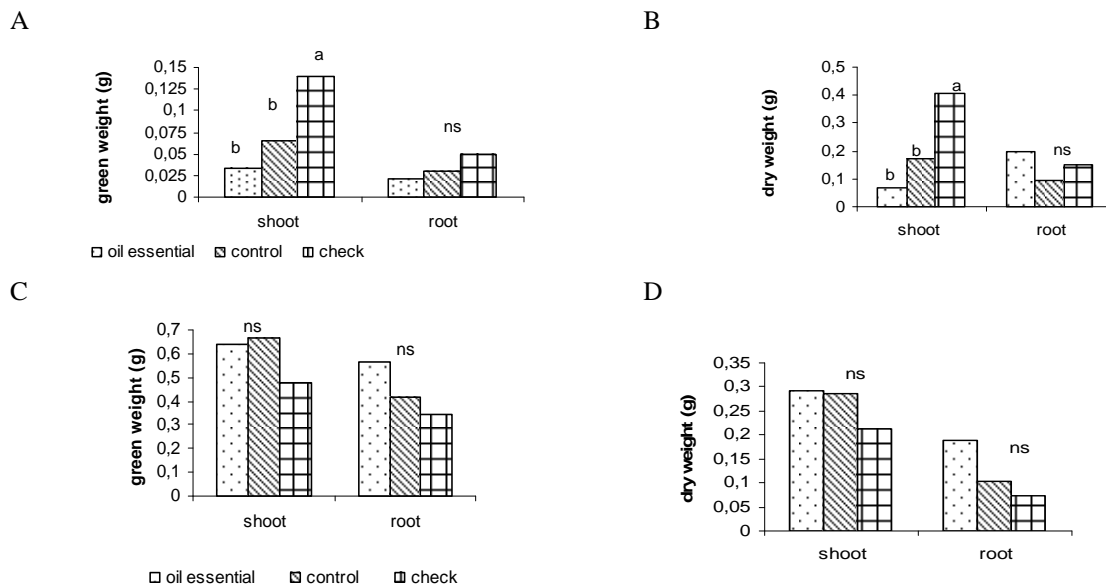


Figure 2 - *E. camaldulensis* oil effect in *Anadenanthera pegrina* green (A) and dry matter mass (B) production 5 and 30 days after the exposition. *Eucalyptus grandis* oil effect in *Anadenanthera pegrina* green (C) and dry matter mass (D) production 30 days after the exposition. (Control= plants incubated without oil; oil= plants incubated with oil, and check = plants not incubated). Means with different letters are significantly different as determined by Tuckey multiple-range test at the 5% confidence level ($P \leq 0.05$). NS: Not significantly different.

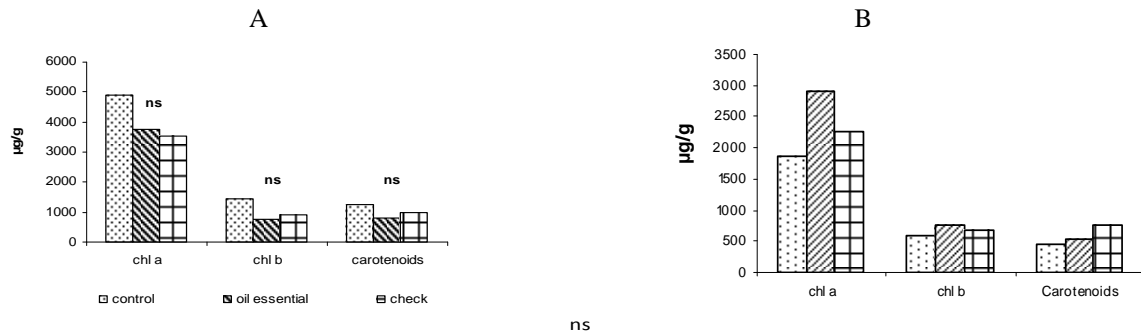


Figure 3 – *E. camaldulensis* oil effect in chlorophyll a, b e carotenoids level in *A. peregrina* leaves (A) after 30 days of exposition. *E. grandis* oil effect in chlorophyll a, b and carotenoids level in *A. peregrina* leaves (B) after 30 days of exposition. (Control= plants incubated without oil; oil= plants incubated with oil, and check = plants not incubated). NS: Not significantly different as determined by Tuckey multiple-range test at the 5% confidence level ($P \leq 0.05$).

Effect of mixed plantation on *A. peregrina* growth

The results confirmed that *A. peregrina* plants were tolerant to *Eucalyptus* spp. effect under the field conditions. *A. peregrina* diameter and height growth (Fig. 4 A and B), did not differ significantly between the single and mixed stands of *E. camaldulensis* plus *M. urundeuva*.

In the study conducted by Scotti and C rrea (2004) in the Legal Reserve of Ja ba-Minas Gerais, the plants of *A. peregrina* which were

inoculated with *Rhizobia* and associated with AM, showed a significant increment in height and total nitrogen content over that non-inoculated plants. The growth in height and diameter in the *E. camaldulensis* in treatment 5 was larger where this was in consortium and received the inoculation showing, thus, the inoculation benefit for *E. camaldulensis* (Fig. 5).

The success of field results clearly confirmed the tolerance of *A. peregrina* to allelopathic effect of essential oil of *E. camaldulensis*.

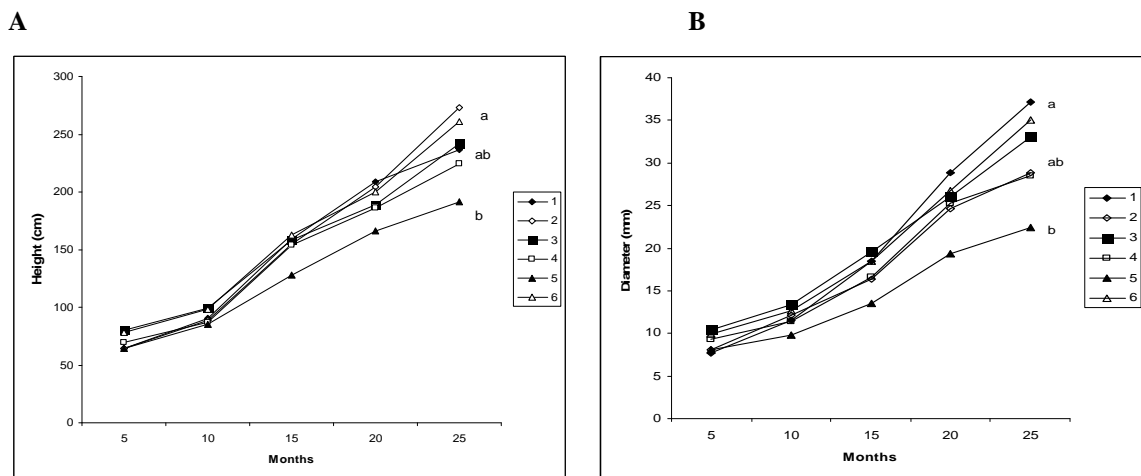


Figure 4 – Effect of intercropped plantation on height (A) and diameter (B) growth of *A. peregrina* under different treatments: 1- Single plantation of *A. peregrina*, 2- Single plantation of *A. peregrina* inoculated with *Rhizobia* and mycorrhizal fungi (AMF). 3- Mixed plantation of *A. peregrina* + *E. camaldulensis* + *M. urundeuva*. 4- Mixed plantation of *A. peregrina* (*Rhizobia* + AMF) + *E. camaldulensis* + *M. urundeuva*, 5- Mixed plantation of *A. peregrina* + *E. camaldulensis* (AMF) + *M. urundeuva* 6- Mixed plantation of *A. peregrina* (*Rhizobia* + AMF) + *E. camaldulensis* (AMF) + *M. urundeuva*. Means with different letters are significantly different as determined by Tuckey multiple-range test at the 5% confidence level ($P \leq 0.05$).

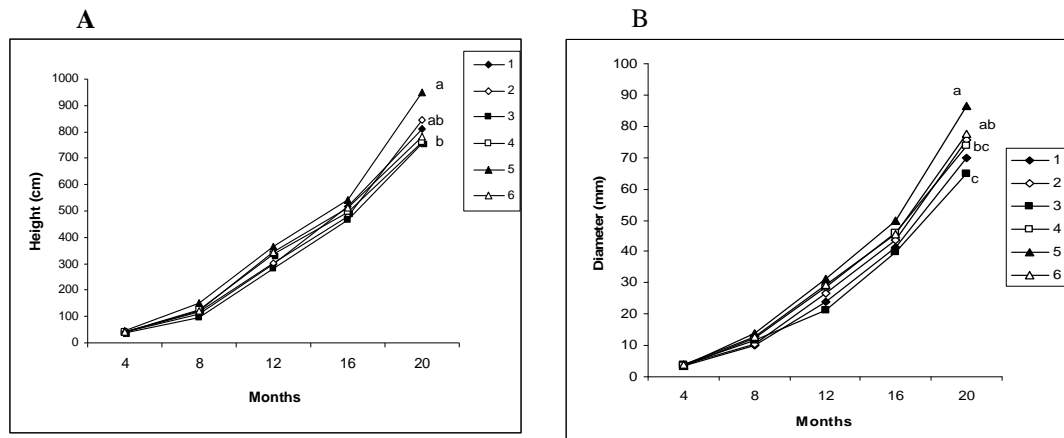


Figure 5 – Effect of intercropped plantation on height (A) and diameter (B) growth of *E. camaldulensis* under different treatments: 1- Single plantation of *E. camaldulensis*, 2- Single plantation of *E. camaldulensis* + AMF 3- Mixed plantation of *A. peregrina* + *E. camaldulensis* + *M. urundeuva*. 4- Mixed plantation of *A. peregrina* (Rhizobia + AMF) + *E. camaldulensis* + *M. urundeuva*, 5- Mixed plantation of *A. peregrina* + *E. camaldulensis* (AMF) + *M. urundeuva* 6- Mixed plantation of *A. peregrina* (Rhizobia + AMF) + *E. camaldulensis* (AMF) + *M. urundeuva*. Means with different letters are significantly different as determined by Tuckey multiple-range test at the 5% confidence level ($P \leq 0.05$).

There are reports that some species are more tolerant than others to the inhibitory effects of *Eucalyptus* spp. oil over germination and plant growth (Kohli and Singh 1991), depending on the physiological and biochemical proprieties of the plants (Hasegawa et al. 1992, Suzuki et al. 2001; Iqbal et al. 2002). The similar occurred in the present study, which showed the high tolerance of *A. peregrina* plants to *E. grandis* and *E. camaldulensis* oils. Several authors have attributed the natural allelopathic effect in the native forest, as an important control mechanism (Mallik 1992), modeling the plant community structure and their composition (Fischer 1986; Mizutani 1989; Seigler 1996). Under the conditions, the *Eucalyptus* species showed prejudicial effects for the adjacent cultures (Jensen 1983; Onyewotu 1985; Igbanugo 1988a, b; Kohli 1990; Mallik and Sharma 1990; Puri and Bangawa 1992; Singh and Kohli 1992). However, Duarte et al, (2006) found that *E. contortisiliquum* plants could be intercropped with *E. grandis* for reforestation in agroforestry systems under the same field conditions of the current study. Similarly, the *E. contortisiliquum* growth was not inhibited when intercropped with *E. grandis*.

Scotti and C rrea (2004) confirmed the positive effect of the double inoculation on the growth of

A. peregrina. Also, they found that in plots where *A. peregrina* was inoculated, the growth and survival of intercropped plants as *Myracrodruon urundeuva* were favored. Chaves et al. (2006), confirmed the benefit of inoculation for *A. peregrina* growth.

The literature shows that soil microorganisms population may be also inhibited by allelopathic effect of *Eucalyptus*. Inhibition of soil bacteria (DELLA BRUNA et al. 1989) or nitrogen fixing bacteria with reduction of *Leucaena leucocephala* nodulation (Moura al. 1996) were registered. However, tolerant rhizobia strains were selected. DUARTE et al, 2006 also showed that inoculated rhizobia strain for *E. contortisiliquum* was also tolerant to allelopathic effect of *E. grandis*. Similarly, the inoculated strain BHICB-A-10 appeared to be tolerant to *E. camaldulensis* effect since the growth of all inoculated plants (treatments 2 and 6) were higher than non-inoculated plants (treatments 1 and 5) as showed in Figure 4.

However, the literature shows that soil microorganisms may be inhibited by the allelopathic effect of *Eucalyptus*. As well as the arbuscular mycorrhizal fungi (AMF) can promote the plant tolerance to heavy metals and have been indicated for use in revegetation processes of the

soils impacted by copper mining (Lins et al. 2007). Besides to the tolerance to *E. camaldulensis* oil, the strain BHICB -A10 revealed to be an efficient nitrogen fixing strain as demonstrated by Pagano et al. 2008 in the same experiment in semiarid. These authors demonstrated the advantage of the double inoculation of rhizobia and AMF to *A. peregrina* growth through the improvement of dry matter, plant nutrient and especially total N content.

In this *in vitro* assays, the oil extracted from *E. camaldulensis* and *E. grandis* leaves did not show inhibitory effect on the growth *A. peregrina*. Under the field conditions, *A. peregrina* could be cultivated in mixture with *E. camaldulensis*, especially when inoculations of both the species were done.

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