Industrial Crops and Products 76 (2015) 734–738

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Essential oil content and chemical composition of *Cymbopogon citratus* inoculated with arbuscular mycorrhizal fungi under different levels of lead

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ARTICLE INFO

Article history:
Received 4 March 2015
Received in revised form 21 May 2015
Accepted 9 July 2015
Available online 13 August 2015

Keywords:
Mycorrhiza
Aromatic plants
Lemon grass
Heavy metals
Citral
Geranial

ABSTRACT

Lemon grass, *Cymbopogon citratus* Stapf, is an important aromatic plant used by industries that produce fragrances and aromas. This topic has been investigated very little in terms of arbuscular mycorrhizal fungi (AMF) associations and the effects of heavy metals, such as lead (Pb), in its metabolism. This study aimed to evaluate the essential oil (EO) content and the chemical composition obtained from lemon grass cultivated with or without AMF *Rhizophagus clarus* inoculums under five levels of Pb in the soil. The experiment was conducted in a greenhouse for six months and consisted of a completely randomized design. A 5×2 factorial was used for five levels of Pb (0, 50, 100, 500, and 1000 mg Pb kg^{-1} soil) and two levels of AMF inoculation (with or without *R. clarus*) and five replicates, totaling 50 experimental units. The EO content was determined and the chemical composition analyzed by GC and GC/MS. The secondary metabolites were affected by the presence of Pb in the soil as well as by the AMF association, which altered the content and chemical composition of EO. The levels of 500 and 1000 mg Pb kg^{-1} soil together with AMF association increased EO content up to 0.69%. In total, 21 components of EO were identified, and along the increasing levels of Pb, the plant metabolism changed and altered the major components of EO. Without AMF inoculation, the major constituent of EO was citral, with concentrations ranging from 36.66 to 45.08% for the lowest levels of Pb. With AMF inoculation, EO was composed mainly of geranial, with concentrations ranging from 39.27 to 58.97% for all Pb levels. Without AMF inoculation, the concentrations of geranial ranged from 43.66 to 62.95% for the highest levels of Pb. High levels of citral and geranial are of great interest to the aroma and fragrance industry, and citral is a basic substance for the synthesis of vitamin A and ionone.

1. Introduction

Among the plant families that produce essential oils (EOs), Poaceae is one of the largest families, comprising approximately 500 genus and 8000 herb species generally known as *grasses*. *Cymbopogon citratus* Stapf originated from India and belongs to the Poaceae family (Barbosa et al., 2008). This plant is widespread throughout the world and is easily found in tropical countries such as Brazil. It is popularly known as citronella grass or lemon grass. This plant has been highly valued in the pharmaceutical, aromatic, fragrance and food industries due to the high content of EO in its leaves. The major components of EOs obtained from lemon grass are the neral, citral and geranial, along with other components that are found in lower amounts. These major components show sedative, diuretic, analgesic, vermicide, insecticide, larvicide and anti-microbial effects (Barbosa et al., 2008; Andrade et al., 2009).

The environment in which the plants develop can redirect the metabolic pathway, causing the biosynthesis of different compounds that may affect the EOs. These environmental changes include: biotic and abiotic factors such as plant age and development stage, luminosity, temperature, rainfalls, soil fertility, day and time of harvest, techniques of collection and processing, level of

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http://dx.doi.org/10.1016/j.indcrop.2015.07.009
0926-6690/

pollution and soil microbial interactions such as arbuscular mycorrhizal fungi (AMF) (Morais, 2009; Santos et al., 2009; Lermen et al., 2015; Urcoviche et al., 2015).

Among various types of pollution, the soil contamination by heavy metals (HMs), in most cases, causes negative consequences to the growth and development of plants. Therefore, it prejudices and changes the production of active compounds (Lermen et al., 2015; Sá et al., 2015). Soil contamination caused by HMs has increased mainly as a result of mining and manufacturing activities, the use of sewage sludge and the application of fertilizers or pesticides in rural areas. In addition to reducing plant productivity, the accumulation of HMs in soils can indirectly affect both human and the animal health (Souza et al., 2012; Sá et al., 2015).

The remediation process of HMs, such as lead-(Pb) contaminated soils, is significantly expensive for many industries and government bodies (Punamiya et al., 2010). In light of this, researchers have investigated alternatives that help to reverse this situation by using species of soil microorganisms like AMF, which are able to alter both the bioavailability of HMs and the plant response to their presence in soils (Audet and Charest, 2007; Wang et al., 2012).

Few studies have demonstrated that AMF symbiosis can contribute to plant tolerance of HMs; however, such mechanisms are not totally elucidated (Wang et al., 2012). Two hypotheses have been proposed to explain the role of AMF symbiosis in the phytoremediation of soils containing HMs: the increased extraction of HMs by the fungal hyphae or the increased plant tolerance to the HMs due to their lower bioavailability caused by the chemical bonds that are established between fungi and metals (Audet and Charest, 2007).

Lead is generally toxic HM that has low solubility in soils. The main visual indicator of Pb toxicity in plant is growth reduction followed by structural alterations that affect the content and quality of the EO (Lermen et al., 2015; Sá et al., 2015). In this context, the present study aimed to evaluate the content and chemical composition of EOs from lemon grass plants (whether inoculated or not) with AMF *Rhizophagus clarus* under five levels of Pb in the soil.

2. Materials and methods

2.1. Experiment design and set up

The soil for the experiment was collected at 0–20 cm depth in the experimental field of the Paranaense University—UNIPAR, Umuarama city, Paraná State, at coordinates S 23° 46′ 11.34″ and WO 53° 16′ 41.78″ and at 391-m height from sea level. A soil sample was subjected to chemical characterization at "Solo Fétil" laboratory (Table 1).

The soil was passed through a 0.4 mm sieve, and then 15 kg soil was placed into two dark polyethylene bags for fumigation with chloroform at 10 mL ($CHCl_3$) kg^{-1} soil (Endlweber and Scheu, 2006). After mixing the soil with chloroform, the bags were sealed and left for three days of fumigation. Then, the bags were opened in an exhaustion chamber where, they rested for one week before the experiment took place.

Approximately, 20 cm seedlings of lemon grass were collected at the Medicinal Garden of the Paranaense University. The seedlings were washed in water and disinfected in 70% alcohol for 1 min, and one seedling was planted per pot.

All experimental units, with or without AMF inoculation, were cultivated in 6 L pots with fumigated soil, some containing Pb $(NO_3)_2$ and some not, according to their respective treatments. All plants were grown in a greenhouse for six months.

To obtain 1000 mg Pb kg^{-1} of soil, we weighed 1614.4 mg $Pb(NO_3)_2$ kg^{-1} of soil and used the proportional rates of 0, 50, 100, and 500 mg Pb kg^{-1} (Lermen et al., 2015; Sá et al., 2015). Once weighed, the $Pb(NO_3)_2$ was dissolved in 100 mL of deionized water (Sá et al., 2015), and this solution was mixed with the fumigated soil. The lemon grass seedlings were transplanted 15 days later, after the stabilization of Pb in the soil.

Equivalent amounts of urea-nitrogen were applied to all treatments in order to avoid the undesirable effects of N as $Pb(NO_3)_2$. To balance NO_3 in the control treatment, 292.7 mg Urea kg^{-1} soil was added to have the same amount of total N compared with 1000 mg Pb kg^{-1} soil treatment. In the other Pb treatments, we followed the same procedure to replace the comparable amount of NO_3.

The AMF *R. clarus* soil inoculums from the Glomales bank of UNIPAR Access No. 10 (Lermen et al., 2015; Urcoviche et al., 2015) were applied to the upper third of each pot designed for the AMF treatment. For each pot, 200 g soil inoculum containing 500 spores and infective propagules was added. The control treatments used 100 mL of the filtered soil inoculums (100 g soil inoculum L^{-1} deionized water). Thus, we only have the effect of the inoculated AMF.

All treatments were fertigated every two days with a half concentration of the solution by Hoagland and Arnon, (1950), except for N, which was already applied as $Pb(NO_3)_2$ and urea at the beginning of the experiment.

2.2. Essential oil content

At the end of the vegetative cycle, the lemon grass plants were harvested early in the morning (from 7:00 to 10:00 am) and then separated into aerial and root portions.

The plants were obtained from all treatments and analyzed. From each treatment, 100 g of the fresh aerial parts of the lemon grass plants were submitted to hydrodistillation (with 1 L deionized water) in a modified Clevenger apparatus. The distillation time was 2 h. The EO was removed with hexane, filtered with anhydrous Na_2SO_4 and stored in amber flasks at 4 °C (Santos et al., 2009). The content (%) was obtained after the solvent evaporation.

2.3. Chemical identification of essential oil by GC/MS

The chemical identification of the EO was made by GC–MS, using an Agilent 5973 Network Mass Selective Detector. The capillary column was the DB-5 (5% phenyl–methylsiloxane, 30 m × 0.25 mm id, 0.25 μm). The detection system was the electronic impact on the "Split" mode 2:1 mL min^{-1}. The column temperature was initially programmed at 40 °C, heating at 8 °C min^{-1} to reach the final temperature of 300 °C. The injector and detector temperatures were 250 °C and 320 °C, respectively. Helium was used as a carrier gas at flow rate of 4.8 mL min^{-1}. The amount of injected sample was 1 μL.

2.4. Statistical analyses

The statistical design was completely randomized in 5 × 2 factorial: 5 levels of $Pb(NO_3)_2$ (0, 50, 100, 500, and 1000 mg kg^{-1} soil) and two levels of inoculation (with and without AMF) with five replicates.

The EO content data were subjected to an analysis of variance (ANOVA) using a general linear model with mixed-effects and balanced design. Prior to ANOVA, the Levene's test was applied to data for homogeneity. Means were compared by Tukey's test ($p \leq 0.05$), using the SPSS statistical package, version 16.0 for Windows (SPSS Inc., Chicago, IL, USA).