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Growth of lemongrass (*Cymbopogon citratus* (DC) Stapf) inoculated with arbuscular mycorrhizal fungi (*Rhizophagus clarus* and *Claroideoglomus etunicatum*) under contrasting phosphorus levels

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Abstract

Cymbopogon citratus (DC) Stapf (lemongrass) has many industrial applications. Various factors affect the metabolism of this plant. Specifically, its association with arbuscular mycorrhizal fungi (AMF) increases water absorption and nutrient uptake, mainly phosphorous (P). This study aimed to assess the AMF symbiotic effects on nutrient uptake and growth in *C. citratus* inoculated with the *Rhizophagus clarus* and *Claroideoglomus etunicatum* AMF under different levels of P applied to the substrate. The treatments were prepared in a sterile substrate with high (200 mg kg^{-1}) and low (20 mg kg^{-1}) P levels, with and without AMF inoculation. The experiment was conducted in a greenhouse for six months. A 3 x 2 (3 mycorrhizal x 2 levels of P) factorial experiment in a completely randomized design (eight repetitions) was used in this study. The variables spore density and root AMF colonization, basal soil respiration, microbial biomass carbon, shoot, root and total dry mass, nitrogen (N) and P content in the plant were analyzed. No significant AMF symbiotic effects ($p > 0.05$) on lemongrass growth were observed for the P levels. P and N content in the shoots, and total P, increased in treatments with AMF and at the high P level, particularly in the inoculation with *R. clarus*. Spore density, root colonization, and microbial biomass carbon were higher with AMF inoculation. It was observed that AMF inoculation increased the content of P and N in *C. citratus*, but did not affect plant growth. AMF inoculation can improve soil quality and, combined with a higher P level, can increase P and N uptake by lemongrass plants, modifying the plant metabolism.

Keywords: Lemongrass; mycorrhiza; symbiosis; medicinal plants.
Abbreviations: AMF_ arbuscular mycorrhizal fungi; BSR_basal soil respiration; K_potassium; MBC_microbial biomass carbon; N_nitrogen; NS_nitrogen in shoot; P_ phosphorous; PR_ phosphorus in root; qCO_2_ metabolic quotient of the soil; RDM_root dry matter; SDM_shoot dry matter; TDM_total dry matter

Introduction

Industrial biotechnology is always searching for plants with therapeutic potential. Medicinal plants are concomitantly used with synthetic drugs in folk medicine, since synthetics are commonly considered costly. The demand for aromatic and medicinal plants is rising, following the consumer trend to preferentially use natural products – either food or pharmaceutical – that have been produced in a sustainable way (Cortez et al., 2015).

Cymbopogon citratus (DC) Stapf (lemongrass) is a medicinal and aromatic plant with therapeutic activity, broadly cultivated and used worldwide (Ekpenyong et al., 2015). *Cymbopogon citratus* belongs to the Poaceae family, and presents long, thin green leaves, in which its therapeutic properties are found.

In addition to its well-known use in treating nervous and stomach disorders, the dehydrated leaves of lemongrass are broadly used in the food industry for manufacturing teas. The essential oil obtained from its leaves is also used as flavoring by the food industry, and as a component of pharmaceuticals, herbal products, insecticides, cosmetics, and perfumes. Among the reported therapeutic properties of lemongrass are its antimicrobial, analgesic, antitumor, insect-repellent, and insecticide actions (Ekpenyong et al., 2015). In addition, this plant is also a source of vitamin A (Gomes and Negrelle, 2015).

The quality of herbal medicines depends on various decisions, mainly the correct identification of a species, crop management, and edaphoclimatic conditions (Lermen et al., 2015, 2017). Inoculation of medicinal and aromatic plants

with arbuscular mycorrhizal fungi (AMF) may increase plant growth and secondary metabolite production, such as essential oils. Mycorrhizal associations can improve plant nutrition, increasing water and nutrient uptake, particularly phosphorus (P) (Urcoviche et al., 2015; Lermen at al., 2017).
Through improving plant nutrition, AMF inoculation usually increases plant growth (Smith and Read, 2008). Mycorrhizal symbioses between fungi and plant occur in a three-phase heterogeneous system, where soil, plant, and fungus are in contact, but their interactions are not mixed. The success of mycorrhizal symbiosis is due to the degree of a plant's mycorrhizal dependence, the fungus' efficiency in absorbing water and nutrients, and the availability of P in the soil. An appropriate level of P in the soil is the main edaphic factor for the success of mycorrhizal symbiosis and positive effects on plant growth (Urcoviche et al., 2015; Lermen at al., 2017).
Treseder (2013) reported that small quantities of P applied to P-poor soils may favor the colonization and sporulation of AMF. Either colonization or sporulation in AMF is inhibited with high doses of P, and the magnitude of this effect depends on the plant species.
P is an essential macro-element in plant nutrition. Many authors have described the importance of P for establishing and developing tropical and temperate crops (Urcoviche et al., 2015; Lermen at al., 2017). P uptake is performed by plant roots, but can also be performed by the external mycelium of AMF, as demonstrated in studies about colonization by *Claroideoglomus etunicatum (= Glomus etunicatum)* in roots of *Lolium perene* (L.) (Smith and Read, 2008).
In this context, this work aimed at assessing the effects of *Rhizophagus clarus* and *C. etunicatum* AMF on soil microbial biomass carbon, soil basal respiration, metabolic quotient, AMF root colonization and spore density, and the growth and accumulation of nitrogen (N) and P in lemongrass plants cultivated in substrates with different P levels.

Results and Discussion

Spore density and root colonization by AMF

AMF inoculation was the treatment that most significantly influenced the studied variables ($p \leq 0.05$), with significant differences observed among seven of the 14 variables (Table 1). P levels influenced five variables. The AMF x P interaction produced significant effects on root colonization, P content in the plant shoot, and total P in the plant.
The *R. clarus* AMF showed the highest spore density in the substrate, followed by the treatment with *C. etunicatum* and 200 mg P kg^{-1}, and finally by *C. etunicatum* with 20 mg P kg^{-1} (Table 2).
The highest AMF root colonization was obtained when *R. clarus* and *C. etunicatum* were associated with low P levels. AMF root colonization and spore density were measured because, according to Smith and Read (2008), the availability of P in soil is the edaphic factor most important for the functioning of mycorrhizal symbiosis.
Urcoviche et al. (2015) investigated the colonization potential of the *C. etunicatum* AMF on *Mentha crispa* L. The authors found an increased colonization of 61.19% when the AMF were combined with 20 mg P kg^{-1} soil, and of 39.32% when the AMF were combined with 200 mg P kg^{-1} soil.
All variables increased when the lemongrass plants were inoculated with AMF (Table 2). Cho et al. (2009) studied AMF colonization in *Panax ginseng* C.A. Meyer, observing an increased density of spores and root colonization when those plants were inoculated with AMF.
Gupta et al. (2002) observed an increased AMF root colonization in *Mentha arvensis* when the plant was inoculated with *Glomus fasciculatum,* similar to the results of this study.

Soil microbiological parameters

The microbial biomass carbon (MBC) was affected by AMF inoculation, with significant increases being observed (Table 3); however, for qCO_2, significant decreases were associated with AMF inoculation. Both the increased MBC and decreased qCO_2 suggest a soil carbon sequestration preventing loss to the atmosphere, increasing soil quality, and consequently maintaining the sustainability of the system (Kaschuk et al., 2010). The basal substrate respiration (BSR) did not differ significantly among treatments.

Biomass production by the plant and agronomic characteristics

Plant height, SDM, RDM, TDM, and SDM/RDM were not significantly affected by the treatments (Tables 1 and 4).
Lermen et al. (2015), studying the growth of *C. citratus* inoculated with AMF, and with different levels of lead, observed increases in plant biomass production with AMF inoculation compared to the control. Urcoviche et al. (2015) also found no significant differences in SDM, TDM, and plant height in *M. crispa* that was not inoculated with AMF. Oregano (*Origanum onites* L.) and mint (*Mentha requienii* Bentham) have produced 2 to 4.7 times dry mass when inoculated with AMF (Karagiannidis et al., 2011); the same response was also observed by Khaosaad et al. (2006) in oregano.

P content in the plant and N content in the shoots

The N content in the shoots was increased with inoculation with *R. clarus* and *C. etunicatum* (Table 5). The P content in the shoots was similar among the four AMF treatments and the control with high P level (Table 5). The P content in the roots and P total were increased in treatments with *R. clarus* AMF and a high P level.
Increases in nutrient uptake, especially P, are considered to of primary benefit to plant nutrition. Commonly, the AMF benefits to plant growth are replaced by the application of nutrients, mainly P (Clark and Zeto, 2000).
Khaosaad et al. (2006) reported that the P content in leaves of *Origanum vulgare* L. was increased with AMF colonization, in comparison to non-inoculated plants; however, the highest content of P in the plants was observed in treatments with added P in the soil, without AMF colonization.
In this study, the *R. clarus* and *C. etunicatum* AMF and P levels (20 and 200 mg kg^{-1} substrate) differentially influenced the variables and parameters analyzed.

Table 1. *p*-Values (in bold are significant) for two-way ANOVA of various soil parameters and plant variables of *Cymbopogon citratus* growing under low and/or high levels of P (mg kg^{-1} substrate) and inoculated or not with AMF.

Soil parameters/Plant variables	AMF	P	AMF x P
Spores (no. of spores g^{-1} dry substrate)	<0.001	0.481	0.538
Root colonization by AMF (%)	<0.001	<0.001	0.043
(MBC) Microbial biomass carbon (µg CO_2 g^{-1})	<0.001	0.374	0.340
(BSR) Basal substrate respiration (µg C-CO_2 g^{-1} h^{-1})	0.856	0.593	0.218
Metabolic quotient (µg CO_2 $µg^{-1}$ C-microbial h^{-1})	0.024	0.912	0.353
(SDM) shoot dry matter (g)	0.278	0.860	0.389
(RDM) root dry matter (g)	0.791	0.688	0.540
(TDM) total dry matter (g)	0.288	0.990	0.288
SDM/RDM	0.557	0.398	0.833
Plant height (cm)	0.123	0.767	0.421
N content in the plant shoot (mg kg^{-1})	0.011	0.004	0.096
P content in the plant root (mg kg^{-1})	0.245	0.015	0.749
P content in the plant shoot (mg kg^{-1})	0.008	0.001	0.001
Total P content (mg kg^{-1})	0.012	<0.001	0.009

Table 2. Root colonization (%) by mycorrhizal fungi (AMF) and spore density (no. of spores g^{-1} dry substrate) of *Cymbopogon citratus* growing under low and/or high levels of P (mg kg^{-1} substrate) and inoculated or not with AMF.

Treatments	Spores	Colonization
Control low-P	0.33±0.05c	1.77±0.48c
Control high-P	0.32±0.05c	1.48±0.49c
R. clarus and low-P	4.44±0.61a	20.92±1.47a
R. clarus and high-P	4.40±0.67a	15.44±1.16b
C. etunicatum and low-P	3.03±0.39b	21.75±1.09a
C. etunicatum and high-P	3.81±0.23ab	17.95±1.01b

Mean values (n = 8, ±standard error); different letters in the same column differ significantly by the Duncan test ($p \leq 0.05$).

Table 3. Values of microbial biomass carbon (MBC, µg CO_2 g^{-1}), basal substrate respiration (BSR, µg C-CO_2 g^{-1} h^{-1}) and metabolic quotient (qCO_2, µg CO_2 $µg^{-1}$ C-microbial h^{-1}) of substrate with low and/or high levels of P (mg kg^{-1} substrate) cultivated with *Cymbopogon citratus* and inoculated or not with AMF.

Treatments	MBC	BSR	qCO_2
Control low-P	137.39±19.59c	1.16±0.12^{ns}	10.47±2.23ab
Control high-P	158.72±21.07bc	1.44±0.19	13.19±5.34a
R. clarus and low-P	166.61±20.63bc	1.36±0.21	9.33±2.19ab
R. clarus and high-P	242.49±33.52ab	1.07±0.12	4.18±0.70b
C. etunicatum and low-P	318.51±45.49a	1.09±0.15	3.95±0.65b
C. etunicatum and high-P	294.98±47.57a	1.34±0.24	5.04±1.15b

Mean values (n = 8, ±standard error); different letters in the same column differ significantly by the Duncan test ($p \leq 0.05$). NS: not significant.

Table 4. Plant height (cm), shoot dry matter (SDM, g), root dry matter (RDM, g), total dry matter (TDM, g), shoot dry matter and root dry matter ratio (SDM/RDM) of *Cymbopogon citratus* growing under low and/or high levels of P (mg kg^{-1} substrate) and inoculated or not with AMF.

Treatments	Height	SDM	RDM	TDM	SDM/RDM
Control low-P	1.16±0.04^{ns}	37.08±2.87^{ns}	11.78±1.13^{ns}	48.87±2.31^{ns}	3.41±0.40^{ns}
Control high-P	1.12±0.03	43.20±4.53	13.39±2.33	56.60±5.08	3.89±0.58
R. clarus low-P	1.18±0.03	37.28±5.15	12.31±1.03	49.59±5.79	3.02±0.36
R. clarus high-P	1.19±0.04	34.82±2.04	10.69±1.13	45.51±3.12	3.39±0.19
C. etunicatum low-P	1.07±0.04	35.76±2.61	12.56±2.31	48.32±4.39	3.36±0.49
C. etunicatum high-P	1.13±0.03	33.62±2.62	10.92±1.52	44.54±3.51	3.36±0.32

Mean values (n = 8, ±standard error). NS: not significant.

Table 5. Contents of nitrogen (mg kg^{-1}) in the shoot (NS) and phosphorus (mg kg^{-1}) in the root (PR), shoots (PS) and total (Total P) of *Cymbopogon citratus* growing under low and/or high P levels (mg kg^{-1} substrate) and inoculated or not with AMF.

Treatments	NS	PR	PS	Total P
Control low-P	21.37±1.25c	4.38±0.38b	2.62±0.22b	7.00±0.54c
Control high-P	25.20±1.39bc	5.14±0.20ab	5.52±0.44a	10.66±0.55ab
R. clarus low-P	23.62±1.19bc	4.74±0.32ab	5.05±0.47a	9.79±0.58ab
R. clarus high-P	30.37±1.57a	5.51±0.32a	5.71±0.34a	11.22±0.32a
C. etunicatum low-P	27.26±2.04ab	4.44±0.25b	4.91±0.36a	9.35±0.59b
C. etunicatum high-P	27.60±0.95ab	4.80±0.31ab	4.76±0.47a	9.58±0.59ab

Mean values (n = 8, ±standard error); different letters in the same column differ significantly by the Duncan test ($p \leq 0.05$).

Table 6. Treatments with or without AMF associated with high or low phosphorous levels applied to fumigated substrate for growing *Cymbopogon citratus* plants.

Treatments	AMF	Phosphorous levels (mg P kg^{-1} substrate)
T1	Non-inoculated	20
T2	Non-inoculated	200
T3	*Rhizophagus clarus*	20
T4	*Rhizophagus clarus*	200
T5	*Claroideoglomus etunicatum*	20
T6	*Claroideoglomus etunicatum*	200

Materials and Methods

Plant material

The 20 cm seedlings were obtained from the young shoots of *C. citratus* in the medicinal garden of the Paranaense University (UNIPAR). After disinfection with 70% alcohol for 1 minute, two seedlings of *C. citratus* were transplanted to each pot.

AMF inoculum

The *C. etunicatum* and *R. clarus* soil inoculums, from the Glomales bank of UNIPAR, were applied to the upper third of each pot designated for AMF treatment, following Urcoviche et al. (2015). *C. etunicatum* was applied at 105 g soil inoculum, and *R. clarus* at 200 g soil inoculum, both containing approximately 200 AMF spores. To the control treatments (without AMF), 100 mL of the filtered soil inoculums was added to each pot. Thus, only the effects of the added AMF were maintained after the soil was inoculated.

Experiment design and set up

The experiment was conducted in the greenhouse of the União de Ensino do Sudoeste do Paraná (UNISEP) in Dois Vizinhos, Paraná, Brazil, at 560 m elevation, from December 2015 to May 2016.

A mixture of 50% commercial organic substrate, 25% sand, and 25% vermiculite was used in the experiment. The material was sieved through 4 mm mesh and placed into hermetically sealed plastic bags for fumigation with 10 mL of chloroform kg^{-1} soil for three days (Endlweber and Scheu, 2006). Three days later, the bags were opened inside a chemical fume chamber, and left to rest for a week before starting the experiment (Lermen et al., 2015).

The treatments were prepared in 48 polyethylene pots containing 3 kg of substrate. Twenty-four pots were prepared with 20 mg P kg^{-1} soil (low P content treatments), and the other 24 pots with 200 mg P kg^{-1} substrate (high P content treatments). To the low P treatments, 0.38 g pot^{-1} potassium chloride (KCl) was added, following Urcoviche et al. (2015). In this way, all treatments had approximately the same concentration of K in the soil, and varied only in their P levels (Urcoviche et al., 2015).

A 3 x 2 factorial experiment (3 treatments of AMF inoculation and 2 levels of P), in a completely randomized design (eight repetitions), was used in this study. The treatments are presented in Table 6.

All pots were irrigated every two days with a 50% concentration of the solution of Hoagland and Arnon (1950), except for P and K.

Spore density and root colonization by AMF

Spores were extracted from subsamples of 10 g substrate, according to the method of wet sieving (0.710–0.053 mm meshes; Gerdemann and Nicolson, 1963), and were centrifuged at 3000 rpm for 3 minutes in water, and then at 2000 rpm for 2 minutes in 50% sucrose. The supernatant was sieved again over 0.053 mm mesh. Then, the AMF spores were transferred to Petri dishes for counting using a stereoscopic microscopic (at 40x magnification).

Fine roots were collected, bleached, acidified, and colored with trypan blue, following Phillips and Hayman (1970). The count of colonized root segments was performed on slides overlain by coverslips (Giovannetti and Mosse, 1980). In total, 100 segments were counted using a stereoscopic microscope (40–100x magnification).

The total root colonization by AMF was transformed by Equation 1 to normalize the data:

$$Col._t = \left(ArcSen\sqrt{Col.(\%)/100}\right)\cdot(180/\pi) \quad \text{Equation (1)}$$

Where, $Col._t$ is the total colonization, *ArcSen* is the inverse of *Sen*, % is percentage, and π is pi.

Determination of microbial biomass carbon, basal respiration, and the metabolic quotient of the soil

Determination of the MBC in the substrate was modified according to the fumigation-extraction method proposed by Vance et al. (1987) and Tate et al. (1988), using 10 g of substrate to which was added 1 mL of ethanol-free chloroform in flasks destined for fumigation. These were then sealed, and stored in a place without light for 24 hours, with temperatures ranging from 25 to 28 °C. After this, the covers were removed from the flasks inside the exhaust chamber, allowing the chloroform to evaporate, as proposed by Witt et al. (2000).

The extracted MBC was estimated by Equation 2:

$$MBC = \frac{(Cf - Cnf)}{Kc} \quad \text{Equation (2)}$$

Where, *Cf* and *Cnf* are the carbon extracted from the fumigated and non-fumigated substrate samples, and *Kc* is a constant value used for all samples, following Hungria et al. (2009). The Kc value used in this study was 0.4, as suggested by Kaschuk et al. (2010).

The BSR was determined according to Jenkinson and Powlson (1976), using 30 g of the substrate sample.
According to Anderson and Domsch, (1993), the metabolic quotient of the substrate (qCO_2) is the ratio of BSR versus MBC (Hungria et al., 2009).

Plant variables

Six months later in the greenhouse, the plants were separated into shoot and root portions. The shoot height (cm) was determined by tape-measure, and then the plants were dried in a forced air laboratory oven at 65 ^oC until constant mass was reached (g). They were then weighed on digital scales for SDM, RDM, and TDM.

Determination of P and N in shoots and roots

To determine P in the shoots and roots, 0.5 g of the respective dry masses were weighed, and placed in a muffle furnace for incineration at 500 ^oC for 3.5 hours. The samples were then cooled, and added to 10 mL of hydrochloric acid (HCl) at 1 mol L^{-1} in crucibles placed on heat plates for exhausting at 70–80 ^oC over 10 minutes to completely solubilize the elements. P was estimated by colorimetry, using ammonium molybdate + ascorbic acid in a spectrophotometer, with a red filter and 660 nm wavelength, as described by Silva (2009).
The N content in the plant shoots was determined by sulfuric acid digestion at 450 ^oC, using the Kjeldahl method of distillation and titration with HCl at 0.05 mol L^{-1}, as described by Silva (2009).

Statistical analysis

The variables for the treatments (AMF and P) were submitted to a two-way analysis of variance (ANOVA) using a general linear model with mixed-effects. Prior to the ANOVA, Levene's test for equality of variances (homogeneity) was performed. The means of treatment were compared by Duncan's test at (p <0.05), using the software SPSS Statistics for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA).

Conclusion

The interaction between the AMF and *C. citratus* was positive. Inoculation with *C. etunicatum* AMF, combined with P added to the substrate, increased the substrate MBC and decreased qCO_2, thus improving the soil quality. Inoculation with *R. clarus* AMF, combined with P added to the substrate, increased the P and N content in *C. citratus*, but did not interfere with plant growth.

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References

Anderson T-H and Domsch KH (1993) The metabolic quotient for CO_2 (qCO_2) as a specific parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biol Biochem. 25: 393-395.

Cortez LER, Yamaguchi MU, Cortez DAG, Pesco DCS (2015) Avaliação da atividade antifúngica dos óleos essencias de *Lippia alba* (Mill.) N. E. Brown (Verbenaceae) e *Cymbopogon citratus* (D.C.) Stapf (Poaceae). Mundo Saúde. 39: 433-440.

Cho EJ, Lee DJ, Wee CD, Kim HL, Cheong YH, Cho JS, Sohn BK (2009) Effects of AMF inoculation on growth of *Panax ginseng* C.A. Meyer seedlings and on soil structures in mycorrhizosphere. Sci Hortic. 122: 633-637.

Clark RB, Zeto SK (2000) Mineral acquisition by arbuscular mycorrhizal plants. J Plant Nutr. 23: 867-902.

Ekpenyong CE, Akpan E, Nyoh A (2015) Ethnopharmacology, phytochemistry, and biological activities of *Cymbopogon citratus* (DC.) Stapf extracts. Chin J Nat Med. 13: 321-337.

Endlweber K, Scheu S (2006) Establishing arbuscular mycorrhiza-free soil: acomparison of six methods and their effects on nutrient mobilization. Appl Soil Ecol. 34: 276–279.

Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc. 46: 235-246.

Giovannetti M, Mosse BA (1980) Evaluation of techniques for measuring VA mycorrhizal infection in roots. New Phytol. 84: 489-500.

Gomes EC, Negrelle RRB (2015) Análise da cadeia produtiva do capim limão: estudo de caso. Rev Bras Pl Med. 17: 201-209.

Gupta ML, Prasad A, Ram M, Kumar S (2002) Effect of the vesicular – arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* on the essential oil yield related characters and nutrient acquisition in the crops of different cultivar of menthol mint (*Mentha arvensis*) under field conditions. Bioresour Technol. 81: 77-79.

Hoagland DR, Arnon DI (1950) The water culture method for growing plants without soil. Calif Agric Exp Stn. 347: 1-32.

Hungria M, Franchini JC, Brandão-Junior O, Kaschuk G, Souza RA (2009) Soil microbial activity and crop sustainability in a long-term experiment with three soil-tillage and two crop-rotation systems. Appl Soil Ecol. 42: 288-296.

Jenkinson DS, Powlson DS (1976) The effects of biocidal treatments on metabolism in soil–V.A method for measuring soil biomass. Soil Biol Biochem. 8: 209-213.

Karagiannidis N, Thomidis T, LazariD, Panou-Filotheou E, Karagiannidou C (2011) Effect of three Greek arbuscular mycorrhizal fungi in improving the growth, nutrient concentration, and production of essential oils of oregano and mint plants. Sci Hortic. 129: 329-334.

Kaschuk G, Alberton O, Hungria M (2010) Three decades of soil microbial biomass studies in Brazilian ecosystems: lessons learned about soil quality and indications for improving sustainability. Soil Biol Biochem. 42: 1-13.

Khaosaad T, Vierheilig H, Nell M, Zitterl-Eglseer K, Novak J (2006) Arbuscular mycorrhiza alter the concentration of essential oils in oregano (*Origanum sp*., Lamiaceae). Mycorrhiza. 16: 443-446.

Lermen C, Cruz RMS, Souza JS, Marchi BA, Alberton O (2017) Growth of *Lippia alba* (Mill.) N. E. Brown inoculated with arbuscular mycorrhizal fungi with different levels of humic substances and phosphorus in the soil. J Appl Res Med Aromat Plants. 7: 48–53.

Lermen C, Mohr FBM, Alberton O (2015) Growth of *Cymbopogon citratus* inoculated with mycorrhizal fungi under different levels of lead. Sci Hortic. 186: 239-246.

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc. 55: 157-160.

Silva FC (2009) Manual de análises químicas de solos, plantas e fertilizantes. Embrapa informação tecnológica, Brasília, DF. 627p.

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, Academic Press, New York.

Tate KR, Ross DJ, Feltham CW (1988) A direct extraction method to estimate soil microbial-C: effects of experimental variables and some different calibration procedures. Soil Biol Biochem. 20: 329-335.

Treseder KK (2013) The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. Plant Soil. 371: 1-13.

Urcoviche RC, Gazim ZC, Dragunski DC, Barcellos FG, Alberton O (2015) Plant growth and essential oil content of *Mentha crispa* inoculated with arbuscular mycorrhizal fungi under different levels of phosphorus. Ind Crops Prod. 67: 103-107.

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass-C. Soil Biol Biochem. 19: 703-707.

Witt C, Gaunt JL, Galicia CC, Ottow JCG, Neue HU (2000) A rapid chloroform-fumigation extraction method for measuring soil microbial biomass carbon and nitrogen in flooded rice soils. Biol Fertil Soils. 30: 510-519.