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Arbuscular mycorrhizal fungi are more efficient than chemical fertilisers in the production of essential oils of common thyme (*Thymus vulgaris*)

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INTRODUCTION:

Common thyme is an aromatic plant with medicinal, cosmetics and culinary uses. Due to its antimicrobial, antioxidant/antiradical and antitumor activity the essential oils of common thyme are in great demand (Nikolić et al., 2014). A significant part of the production of aromatic plants relies on the use of chemical fertilisers, which when applied in large scale can have significant environmental consequences (Malik et al., 2011). Arbuscular mycorrhizal fungi (AMF) are a group of soil microorganisms that forms mutualistic symbioses that can benefit plants by improving the uptake of mineral nutrients, mineralising organic nutrients and conferring drought resistance (Oliveira et al., 2005).AMF have the potential to be inoculatedin the production of common thyme as an ecotechnological tool to reduce the input of chemicals fertilisers.

OBJECTIVES:

The aim of this study was to assess the effect of inoculation with an arbuscular mycorrhizal fungus, *Glomusintraradices*, and the application of chemical fertilisers on the composition of essential oils produced by common thyme (*Thymus vulgaris* L.).

MATERIALS AND METHODS:

Common thyme plants were propagated through cuttings in trays with 70 cm^3 cells. Half the cells received 5 g of inoculum of *G. intraradices*consisting of spores, mycelia and colonised root fragments. After rooting the plants were transplanted to 1 L pots containing growth substrate. Plants were watered with either deionised water, a low dosage of a soluble chemical fertiliser or a high dosage of a soluble chemical fertiliser. The experiment comprised six treatments: (i) non-inoculated control plants without chemical fertiliser, (ii) non-inoculated control plants with a low dosage of chemical fertiliser, (iii) non-inoculated control plants with a high dosage of chemical fertiliser, (iv) plants inoculated with *G. intraradices*without chemical fertiliser, (v) plants inoculated with *G. intraradices* and a low dosage of chemical fertiliser and (vi) plants inoculated with *G. intraradices* and a high dosage of chemical fertiliser.

After a growth period of 4 months, the essential oils of the leaves of common thymewere isolatedby water distillation using a Clevenger-type apparatus, according to theprocedure described in the European Pharmacopoeia.

Analysis was carried out by gas chromatography (GC) and by gas chromatography-mass spectroscopy (GC/MS). Analytical GC was carried out in a Hewlett-Packard 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph with a HP GC ChemStation Rev. A.05.04 data handling system, equipped with a single injector and two flame ionization detection (FID) systems. A graphpak divider (Agilent Technologies,

part no. 5021-7148) was used for simultaneous sampling to two Supelco (Supelco, Bellefonte, PA, USA) fused silica capillary columns with different stationary phases: SPB-1 (polydimethylsiloxane 30 m × 0.2 0mm i.d., film thickness 0.20 μm), and SupelcoWax-10 (polyethyleneglycol 30 m × 0.20 mm i.d., film thickness 0.20 μm). Oven temperature program: 70-220 ºC (3 ºC.min^{-1}), 220 ºC (15 min); injector temperature: 250 ºC; carrier gas: helium, adjusted to a linear velocity of 30 cm.s^{-1}; splitting ratio 1:40; detectors temperature: 250 ºC. GC-MS was carried out in a Hewlett-Packard 6890 gas chromatograph fitted with a HP1 fused silica column (polydimethylsiloxane 30 m × 0.25 mm i.d., film thickness 0.25 μm), interfaced with an Hewlett-Packard mass selective detector 5973 (Agilent Technologies) operated by HP Enhanced ChemStation software, version A.03.00. GC parameters as described above; interface temperature: 250 ºC; MS source temperature: 230 ºC; MS quadrupole temperature: 150 ºC; ionization energy: 70 eV; ionization current: 60 μA; scan range: 35-350 units; scans.s^{-1}: 4.51.

Essential oil components were identified by their retention indices on both SPB-1 and SupelcoWax-10 columns and from their mass spectra. Retention indices, calculated by linear interpolation relative to retention times of C8-C23 of n-alkanes, were compared with those of reference samples included in the Faculty of Pharmacy, University of Coimbra laboratory database. Acquired mass spectra were compared with reference spectra from the laboratory database, Wiley / NIST library and literature data (Adams, 2004). Relative amounts of individual components were calculated based on GC raw data areas without FID response factor correction.

Fresh root samples were collected and stained as described in Oliveira et al. (2005). Root colonisation by arbuscular mycorrhizal fungi was then assessed by microscopy.

RESULTS AND DISCUSSION:

Both the inoculation with *G. intraradices* and the application of chemical fertilisers influenced the composition of the essential oils produced by common thyme. The application of low and high dosages of chemical fertiliser in non-inoculated plants increased the production of thymol when compared with the plants that did not receive any chemical fertiliser. However, the highest production of thymol was achieved in plants inoculated with *G. Intraradices* without the application of chemical fertiliser, with a 208% increase when compared with the non-inoculated treatment. The highest production of carvacrol was also obtained in plants inoculated with *G. Intraradices* without the application of chemical fertiliser. Thymol and carvacrol are two of the major constituents of the essential oils of common thyme and are known for their antimicrobial and antimutagenic properties (Nikolić et al., 2014). Typical structures of arbuscular mycorrhizas here observed in the roots of inoculated plants, while no colonisation was observed in non-inoculated control plants. The mycorrhizal colonisation was, however, higher in plants without chemical fertiliser. This indicates that the inoculated *G. Intraradices* was capable of colonising the roots of common thyme and that the chemical fertiliser reduced the mycorrhizal colonisation. We hypothesise that the observed increased production of important essential oil components such as thymol and carvacrol was due to the higher efficiency of *G. intraradices* to uptake mineral nutrients in the plants without chemical fertiliser.

CONCLUSION:

The highest contents of thymol and carvacrol, two economically important components of the essential oils of common thyme, were obtained in plants inoculated with *G. Intraradices* without the application of chemical fertiliser. Inoculation with AMF can, therefore, be regarded as an eco-friendly alternative to the application of chemical fertilisers in the production of essential oils of common thyme.

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