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The role of aromatic *Salvia officinalis* L. on the development of two mycorrhizal fungi

Christos N. Hassiotis

Etherio Research Laboratory, Essential Oils, Eratera, 50003, Voio, Greece

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ABSTRACT

The symbiotic associations between plants and arbuscular mycorrhizal fungi (AMF; Glomeromycota) are common in terrestrial ecosystems. AMF stimulate growth, improve pathogen, heavy metal and salinity resistance and influence the content of secondary metabolites in plants. The Mediterranean area is rich in aromatic plants growing naturally. They contain essential oils which will reach on the ground following the litter fall. The aim of this study was to explore the effect of *Salvia officinalis* L. on the development of two mycorrhizal species *Glomus deserticola* and *G. intraradices*. The major compounds of *S. officinalis* essential oil were 1,8-cineole (37.6%) and camphor (23.2%). The level of fungi colonization and the host (*Allium porrum* L.) growth were monitored under different treatments with *S. officinalis* leaves and essential oil. *G. deserticola* and *G. intraradices* colonized successfully the host plants. As a result, the host growth was positively influenced. *G. deserticola* showed higher infection levels and host growth. The addition of *S. officinalis* leaves and essential oil into the soil altered mycorrhiza levels. Small amount of *S. officinalis* leaves (0.75 g L^{-1}) or *S. officinalis* essential oil (11 mg L^{-1}) was beneficial for fungi colonization and host growth. However, fungi inhibition and reduced plant growth was recorded in concentrations over 22 mg L^{-1} (essential oil/soil).

1. Introduction

Mycorrhizas have been studied for over 100 years, but in the last 30 there has been an explosion of interest. Virtually, all plants form mycorrhizal symbioses: probably only 5–10% is non mycorrhizal (Helgason and Fitter, 2005). It is the mutualistic symbiosis (non pathogenic association) between soil-born fungi and roots of higher plants (Frank, 1885) to a single, morphological organ, in which the plant nourishes the fungus and the fungus the plant. About two thirds form a distinctive type of mycorrhiza called arbuscular mycorrhiza (AM), named from the distinctive hyphal structure formed within the cortical cells of roots. Its beneficial effects on the nutrition and development of plants have been clearly shown since the extraradical mycelium surrounds the plant roots (Gosling et al., 2006) and the absorption of the nutritive minerals is more efficient (Schnepf et al., 2011). Inoculation with AMF often facilitates the acquisition of poorly accessible nutrients by plants (Smith et al., 2003; Cavagnaro, 2008; Smith and Read, 2008) and thus promotes their growth (Nell et al., 2010). Inoculation improves water uptake (Miransari, 2014), provides drought and salinity tolerance (Koide and Mosse, 2004; Campanelli et al., 2013), facilitates the accumulation of more dry matter (Sharif and Claassen, 2011), confers protection against pathogens (Filion et al., 1999), influences the qualitative and quantitative profile of secondary metabolites (Zeng et al., 2013) and facilitates nutrient mobility in the soil (Bücking et al., 2015).

Across the Mediterranean region, aromatic plant species growing naturally constitute one of the most important plant categories. They are able to produce and store volatile substances, mainly essential oils that affect living organisms. These compounds are incorporated in plant material and follow the litter fall. Depending on species composition, foliar density, land characteristics, and other factors, plant foliage is the source of at least two-thirds of global VOC (volatile organic compound) emissions (Guenther, 1997). The importance of volatile chemicals within soil and their effect on microorganisms has been established (Joner et al., 2001; Prati and Bossdorf, 2004; Bainard et al., 2009). These compounds alter microbial numbers and their respiration rhythm. In particular, when applied to soil at low concentrations (1 μL g^{-1} of soil) increases microbes whereas at higher concentrations become inhibitory (Bowers and Locke, 2000; Kumbhar et al., 2001; Koide

E-mail address: chasioti@for.auth.gr.

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et al., 2005). They also shift microbial population structure, reducing the number of fungi in favor of bacteria (Letessier et al., 2001; Fujii et al., 2005).

The genus *Salvia* L., one of the largest genera in the Lamiaceae family, comprises over 900 species. The main speciation centers are Oriental Mediterranean, South-West Asia, South Africa and America (Hedge, 1992). In European countries, the genus *Salvia* is represented by 36 species (Hedge, 1972). One of these species, *Salvia officinalis* L., is the most representative within the genus and numerous investigations have been made in this subject and especially on the richness in volatile constituents (Radulescu et al., 2004; Avato et al., 2005; Marić et al., 2006).

There are several studies on the beneficial effects of AMF on aromatic and medicinal plants, such as basil (*Ocimum basilicum* L.) (Pascual-Villalobos and Ballesta-Acosta, 2003; Toussaint et al., 2007), oregano (*Origanum onites* L.) (Khaosaad et al., 2006), mint (*Mentha requienii* Benth.) (Freitas et al., 2004; Cabello et al., 2005), fennel (*Foeniculum vulgare* Mill.) (Kapoor et al., 2004), coriander (*Coriandrum sativum* L.) (Ferahani et al., 2008), lavender (*Lavandula angustifolia* L.) (Tsuro et al., 2001) and sage (*S. officinalis*) (Nell et al., 2009; Tarraf et al., 2017).

On the contrary, the influence of essential oils from aromatic species to/or against mycorrhizal fungi has been woefully under investigated. The aim of the present study was (1) to investigate the way the *S. officinalis* (leaf and essential oil) can affect colonization of two mycorrhizal fungi, (2) to examine the level of mycorrhiza development according to different treatments with *S. officinalis*, (3) to explore the antifungal activity of *S. officinalis* under different treatments with leaves or essential oil into the soil and (4) to monitor the growth in *Allium porrum* L. as host plants in combination with AMF and *S. officinalis* treatments.

2. Materials and methods

2.1. Plant material and substrates

The experiments were conducted using *A. porrum* (leeks) as host plant, because of their relatively good response to mycorrhiza, fast growth, and easy production of plants. The seeds of *A. porrum* were purchased from a specialized store. *S. officinalis* plant material was collected from a native population, near the village of Kato Scholari (N: 40°26′44″, E: 23°01′02″), Thessaloniki, Greece. A voucher specimen (No.100015) was deposited at Etherio Research Laboratory (Voio, Greece), after its identification according to Flora Europaea (Hedge, 1972). The experiments were established in the same place.

2.1.1. Treatments with S. officinalis leaves

Seeds of A. porrum were surface sterilized by soaking in 15% H_2O_2 for 30 min, carefully washed with sterilized water and then germinated in sterile soil (temperature 120 °C, pressure 2 atm for 2 h). The seedlings remained in the germination pots for eight weeks and then were transplanted in new pots (volume 4 L, 4 plants per pot). The potting mixture was made by 6 parts of soil, 9 parts of sand (both sterilized as above) and 15 parts of vermiculite. Five different concentrations of *S. officinalis* mature leaves were used. *S. officinalis* chopped leaves were carefully mixed to the above substrate for each treatment. Those treatments were named as **C** (control): 0 g L^{-1}, **1**: 0.75 g L^{-1}, **2**: 1.5 g L^{-1}, **3**: 3 g L^{-1} and **4**: 6 g L^{-1} (*S. officinalis* leaves per litre of soil). Ten replications (pots) for each treatment were made (50 pots in total with 200 leeks for each AMF).

2.1.2. Treatments with S. officinalis essential oil

The following year, the experiment was repeated in the same way using essential oil instead of *S. officinalis* leaves. The essential oil was extracted from *S. officinalis* of the same native population of the previous experiment. For comparable data purposes, the specific essential oil concentrations were decided following the quantitative estimate in the *S. officinalis* leaves after the first experiment. Those treatments were **C** (control): 0 mg L^{-1}, **1**: 11 mg L^{-1}, **2**: 22 mg L^{-1}, **3**: 44 mg L^{-1} and **4**: 88 mg L^{-1} (per litre of soil). The essential oil was applied in the soil as described by Hassiotis (2010) in 4 L volume cylindrical pots. The essential oil was applied into an absorbent paper stick and then was placed into the tube construction. In order to maintain the concentration of essential oil at specific levels, paper sticks were replaced every two weeks to ensure a constant amount of essential oil in every pot (Hassiotis, 2010; Hassiotis and Dina, 2011). The leek preparation was made as described above.

2.2. Inoculation, root infection and growth parameters

Two arbuscular mycorrhizal fungi were used to establish mycorrhiza with leeks. These were *Glomus deserticola* and *G. intraradices*, both registered in the European Bank of *Glomales* as BEG 73 and BEG 72, respectively. These inoculants were selected because of their growth promoting effect on various plants (Planchette and Fortin, 1982; Furlan et al., 1983), their protective influence against some root pathogens (Caron et al., 1985) and their ability to form abundant internal vesicles (Berch, 1988). The fungi inoculation of the leeks was made at the same time when transplantation has been taken place. During transplantation 16 g of active inoculum were placed in every pot, divided in four inoculation points such as the number of leeks in every pot. 50 pots were used for inoculation with *G. deserticola*, another 50 for *G. intraradices* and 50 more treated without inoculums containing only *S. officinalis* leaves or *S. officinalis* essential oil in different concentrations. The cultures were watered, once a week, using 100 mL of distilled water. At the date of inoculation, and every two weeks afterwards, root colonization, stem diameter and height of leeks were measured and recorded. Four glass slides from every pot, with inoculated leeks, were prepared in order to evaluate root colonization. The host growth was evaluated as average of leek stem diameter and height of leek.

2.3. Mycorrhiza assay

The root samples were extracted by the use of a cylindrical corer (10 mm). The soil was removed by soaking the roots in water and gently washing them, to ensure that all thinner roots and tips remained intact. The staining procedure was performed according to Vierheilig et al. (2005). The roots' infection level was evaluated as described in a previous work (Hassiotis and Dina, 2011).

2.4. Essential oil analysis

Four hundred g of fresh *S. officinalis* from the same population was chopped and steam distilled in a 2 L water steam distillation unit for 90 min, at 100–105 °C and a flow rate of approximately 8.5 mL min^{-1} (Furnis et al., 1989). Eight samples (four in each year) of 400 g of *S. officinalis* were distilled and the obtained essential oils were analyzed twice in order to determine the chemical composition. The amount of oil was expressed (w/w) as essential oil/fresh raw material. The essential oil was collected and its volatile constituents were recognized by GC-MS analysis. GC-MS analysis was performed on a Shimadzu GC-2010 – GCMS-QP2010 system operating in EI mode (70 eV) equipped with a split/splitless injector (230 °C), a split ratio 1/30, using a fused silica HP-5 MS capillary column (30 m × 0.25 mm (i.d.), film thickness: 0.25 μm). The