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Article

Mycorrhizal Inoculation Improves the Quality and Productivity of Essential Oil Distilled from Three Aromatic and Medicinal Plants: *Thymus satureioides*, *Thymus pallidus*, and *Lavandula dentata*

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Abstract: In Morocco, extensive use, traditional practices, and climate change have seriously impacted the productivity of aromatic and medicinal plants (AMP). To mitigate these adverse effects, this study aims at evaluating the potential of the arbuscular mycorrhizal fungi (AMF), namely *Rhizophagus irregularis* and *Funneliformis mosseae*, in improving biomass, essential oils (EOs), and biomolecule production in *Thymus satureioides*, *T. pallidus*, and *Lavandula dentata*. Compared to non-inoculated-AMP, AMF induced significant increases in biomass production by 37.1, 52.4, and 43.6%, and in EOs yield by 21, 74, and 88% in *T. satureioides*, *T. pallidus*, and *L. dentata*, respectively. The EOs of inoculated-AMP exhibited increased proportions of major compounds such as thymol (23.7%), carvacrol (23.36%), and borneol (18.7%) in *T. satureioides*; α -terpinene (32.6%), thymol (28.79%), and δ -terpinene (8.1%) in *T. pallidus*; and camphor (58.44%), isoborneol (8.8%), and fenchol (4.1%) in *L. dentata*. Moreover, AMF significantly improved the anti-germinative and antifungal activities of the EOs. Indeed, IC₅₀ values decreased by 1.8, 16.95, and 2.2 times against *Blumerai graminis*, *Zymoseptoria tritici*, and *Fusarium culmorum*, respectively, compared to non-inoculated-AMP. This study highlights the performance of the symbiosis between AMF and AMPs in terms of high quality of EOs production while respecting the environment. The associations *F. mosseae*-*Thymus* and *R. irregularis*-*Lavandula* are the most efficient.

Keywords: aromatic and medicinal plants; arbuscular mycorrhizal fungi; *Thymus satureioides*; *Thymus pallidus*; *Lavandula dentata*; essential oils; biological activities

1. Introduction

Plants with medicinal and/or aromatic properties that are used in pharmacy and/or perfumery are usually defined as aromatic and medicinal plants (AMP) [1]. Since antiquity, humans have used AMP as a source of therapeutics to remedy ailments, relieve pain, heal wounds, and also in the composition of perfumes and culinary preparations. These uses developed later through civilizations in China, India, and Greece [2]. Backed by natural assets, including its ecological heterogeneity, climatic variations, fertile soils, and geographic location, Morocco has a diversified floristic system which includes more than 4200 wild species, including 800 species with aromatic and/or medicinal properties [3,4]. It is estimated that the area of wild AMP in Morocco exceeds 311,862 Ha [5], but only a hundred species are exploited as dried herbs and as essential oils or other aromatic extracts [6]. The Moroccan production of AMP herbs and their extracts comes from both

wild-crafted and farmed species, providing export revenues of about 25 million dollars for cultivated AMP and 37 million dollars for wild AMP [6]. Morocco is ranked as the 12th exporter worldwide, of which Europe is the leading destination, where AMP are exported as essential oils and dried material [7]. The analysis of AMP exports between 2002 and 2014 highlighted the dominance of thyme and lavender, with an average volume of about 1973.49 tons and 109.17 tons, respectively [8]. These plants, belonging to the Lamiaceae family, possess outstanding biological activities due to their valuable chemical compositions, including terpenes, even though the essential oil compositions of *Lavandula* and *thymus* species show a similar chemical profile for the same genus with a variation of proportion, such as thymol and its isomer carvacrol as well as their precursors p-cymene, γ -terpinene, and borneol in thyme [9–11] and eucalyptol, fenchone, and camphor in lavender [12,13], in addition to linalool, which is common in both plants [10].

However, the method of exploitation of AMP in Morocco has several weaknesses upstream and downstream of the sector. Most biomass collected is spontaneous (more than 80%) rather than the cultivated origin (less than 20%). In addition, most AMP are collected at the flowering stage, compromising their regenerative ability [14,15]. This type of harvest, if not wisely managed, can lead to overexploitation, genetic erosion, and finally, extinction of these resources. The use of chemical fertilizers to improve productivity—regardless of the negative effect on human health and on environmental ecosystems—no longer conforms to the requirements of the world market, which is increasingly interested in bioactive molecules. Many studies have shown that the use of nitrogen-based fertilizers in high doses leads to a decrease of up to two times in secondary metabolites, such as total yields of phenols and flavonoids and the equivalents of ursolic acid, carlina oxide, and chlorogenic acid [16,17]. Thus, sustainable exploitation of AMP must include strategies for improving productivity, producing sufficient quantity and quality as required, as well as mastering the cultivation of AMP species well adapted to the environmental conditions of the region [18]. In this perspective, eco-friendly microorganisms, such as arbuscular mycorrhizal fungi (AMF), could be one of the keys to improve AMP yield in terms of biomass and bioactive molecule production. AMF live in symbiosis with the roots of the majority of plant species [19,20]. They provide vital ecological services, such as improving soil texture and fertility through the production of glomalin as well as increasing water and mineral nutrient supply for the host plants [21]. These modifications strengthen host plants' tolerance to environmental stresses, activating secondary metabolite biosynthesis pathways such as essential oil biosynthesis [22,23].

Many studies have shown that AMF increases biomass and essential oil yield in several AMP, such as *Mentha arvensis* L. [24], *Mentha piperita* L. [25], *Origanum vulgare* L. [26], *Ocimum basilicum* L. [27], *Salvia officinalis* L., and *Thymus vulgaris* L. [28,29]. However, little is known about the influence of AMF inoculation on the accumulation of active phytochemicals in AMP shoots, which are often sought-after products. In particular, no data are available about the effect of arbuscular mycorrhizal inoculation on the chemical composition of essential oil in Moroccan endemic *Thymus* (*Thymus satureioides* and *Thymus pallidus*) and *Lavandula* (*Lavandula dentata*). Those species have huge economic value, but they are unfortunately under threat of extinction [30,31].

Thus, the present paper aimed to evaluate the efficiency of two AMF species (*Rhizophagus irregularis* and *Funneliformis mosseae*) on the yield, composition, and biological activities of essential oils in three AMP species (*T. satureioides*, *T. pallidus*, and *L. dentata*) grown under greenhouse conditions, noting the best-performing AMP–AMF associations.

2. Materials and Methods

2.1. Plant and Fungal Materials

The cultivation of thyme and lavender was carried out from seeds collected from wild plants in the region of Asni for *T. satureioides* (N 31°13'35", W 7°57'38", 17152'') and *T. pallidus* (N 31°15'59", W 7°49'46", 68096'') and from the region of Had draa (N 31°38'59", W 9°36'53", 40132'') for *L. dentata*. Seeds were germinated in sterile peat,

and after one month of cultivation, the thyme and lavender seedlings were transplanted and inoculated (20 g/kg) in pots containing a mixture of sand (2/3) and sterile peat (1/3). The mycorrhizal inoculums tested in our study are based on two different AMF species: *R. irregularis* (Ri) (C. Walker and A. Schüßler, No. BGCBJ09) and *F. mosseae* (Fm) (Gerd. and Trappe, BEG no. 12). They were multiplied in our laboratory as previously described [19,32–35]. The inoculum consisted of 20 g of soil with mycorrhizal root fragments, spores, and hyphae of the respective fungus per pot. The same amount of autoclaved inoculum was added to non-inoculated plants. The inoculum from each AMF possessed similar infective characteristics (75% of infected roots and approximately 20 spores/g of inoculum).

2.2. Experimental Setup

The experimental setup consisted of nine treatments, including three mycorrhizal status (non-inoculated (NI), inoculated with *R. irregularis* (Ri), inoculated with *F. mosseae* (Fm)) and three AMP species (*T. saturoioides* (Ts), *T. pallidus* (Tp), and *L. dentata* (Ld)). The 9 treatments (Ts-NI, Ts-Ri, Ts-Fm, Tp-NI, Tp-Ri, Tp-Fm, Ld-NI, Ld-Ri, Ld-Fm) were repeated 42 times (a repeat is represented by a pot containing one plant).

2.3. Determination of Arbuscular Mycorrhizal Colonization and Plant Growth

Evaluation of the mycorrhizal colonization intensity (%) of the roots was performed according to the method of Trouvelot et al. [36], after staining the roots as described by Philips and Hayman (1970) [37] with some modifications. Roots were harvested, washed thoroughly with running water, and then thinned with 10% KOH for 30 min at 90 °C. Excess KOH was removed by rinsing with running water. The thinned roots were then immersed in a solution of trypan blue (0.05%) diluted in Lacto-glycerol (1/3 water, 1/3 glycerol, and 1/3 lactic acid) for 20 min at 90 °C. After a final rinse, the roots were cut into 30 fragments of 1 cm and observed under a light microscope.

Each observed root fragment was assigned a class score between 0 and 5, corresponding to the estimated proportion of cortex colonized by the mycorrhizal symbiot.

$$\text{Colonization intensity (\%)} = (95 \times n_5) + (70 \times n_4) + (30 \times n_3) + (5 \times n_2 + n_1)/N$$

where n_5 , n_4 , n_3 , n_2 , and n_1 are the number of fragments noted as 5, 4, 3, 2, and 1, respectively.

2.4. Determination of Shoot Dry Matter

After four months of cultivation under greenhouse conditions, shoot biomass accumulation expressed as shoot dry matter (SDM) was measured. The plant was dried at 70 °C for 48 h, and the SDM was measured for each treatment and species of AMP.

2.5. Total Chlorophyll, Soluble Sugar, and Protein Content

The determination of total chlorophyll was performed by colorimetry according to the method of Arnon [38]. Briefly, 50 mg of fresh material was ground in 2 mL of acetone (80%) in the dark. After centrifugation at 5000 rpm for 10 min, the optical density (OD) of the recovered supernatant was subsequently determined using a spectrophotometer at wavelengths 645 nm and 663 nm.

The following formula was used to calculate the chlorophyll content:

$$\text{Total chlorophyll} = 20.2 \times \text{DO } 645 \text{ nm} + 8.02 \times \text{DO } 663 \text{ nm}$$

The determination of soluble sugars was carried out according to Dubois et al. [39]. Extraction was performed by cold grinding 50 mg of MF in 4 mL of ethanol (80%). After centrifugation at 5000 rpm for 10 min, we recovered the supernatant, and the pellet was taken up in 2 mL of 80% ethanol and centrifuged again; this second supernatant was added to the first.

In test tubes, 1 mL of a 5% phenol solution and 5 mL of concentrated sulfuric acid were added to 1 mL of supernatant. After vortexing, the tubes were allowed to cool for 5 min, and then the OD was read at 485 nm. The soluble sugar content was determined by reference to a standard range established by known glucose concentrations.

2.6. Nitrogen Content

Total nitrogen content was determined by the method of Kjeldahl [40]. In Matras tubes, 0.5 g of dry biomass was added to 1 g of catalyst (5 g of K_2SO_4 , 0.5 g of $CuSO_4$ and 0.25 g of Se) and 5 mL of concentrated sulfuric acid (H_2SO_4), which was then digested for 2 h at 400 °C. Distillation was performed using boric acid (10 mL) and sodium hydroxide (NaOH) (20 mL). The total concentration of N was determined by titration of 5 mL of the distillate with sulfuric acid (0.02 mol/L) using bromocresol green and methyl red as color indicators.

2.7. Phosphorus and Potassium Content

Dry matter (0.5 g) from each sample was incinerated at 600 °C for 6 h. The ash was recovered in 3 mL of 10 N hydrochloric acid. Then, the extracts were filtered and the filtrate was adjusted to 50 mL with distilled water. The phosphorus (P) content was determined according to the method of Murphy and Riley [41]. Then, 1 mL of the filtrate was mixed with 4 mL of distilled water and 5 mL of reagent AB (sodium molybdate (2.5%) and hydrazine sulfate (0.15%); 2/1 v/v). After incubation in a water bath at 95 °C for 10 min, the absorbance was measured at 825 nm. A standard range was prepared by KH_2PO_4 . The potassium (K) content was determined by flame spectrophotometry [42]. The standard range was prepared using solutions of well-defined concentrations of K^+ . The results are expressed as mg of phosphorus or potassium/g of dry matter.

2.8. Extraction of Essential Oils

For the extraction of essential oils, the aerial part of thyme and lavender was harvested and air-dried in the shade at room temperature. The extraction of essential oil was carried out by hydro-distillation with a Clevenger of 30 g of dried plant material for 5 h. The obtained essential oils were dried with anhydrous sodium sulfate and then stored at 4 °C in the dark until analysis. The extraction was performed three times (3×30 g). The essential oil yield was calculated as $\mu\text{L/g}$ of DM using the following equation:

Yield of the essential oil = (weight of the extracted essential oil/weight of the dried material) \times (1/essential oil density).

2.9. Determination of the Essential Oil Chemical Composition

The essential oil was diluted in acetate ethyl (ratio of 1:200 v/v) and then analyzed by gas chromatography–electron ionization mass spectrometry (QP 2010 Ultra, Shimadzu, Marne-la-Vallée, France). The system was operated using helium as a carrier gas at a constant linear velocity (60 cm/s).

Briefly, 0.2 μL of the resulting already-diluted essential oil was injected in split mode (split ratio of 1:10 at a temperature of 260 °C) into a ZB-5MS capillary column (5% phenylacetlene, 95% dimethylpolysiloxane; 10 m length, 0.10 mm inner diameter, 0.10 μm phase thickness; Phenomenex, Le Pecq, France). The column temperature was programmed to increase linearly from 60 °C (for 2 min) to 280 °C (for 1 min) at a constant rate of 40 °C/min.

Mass spectra were recorded with an ionization energy of 70 eV and an interface temperature of 280 °C over a mass range from 35.0 to 350 (m/z). The identification of essential oil compounds was performed by comparing the Kovats indices obtained from retention times and after co-injection of n-alkanes with those found in the literature and The Pherobase: Database of Pheromones and Semiochemicals. The relative percentages of the constituents of the essential oils were calculated from the area under the peak obtained from the GC-FID chromatogram.

2.10. Biological Properties of Essential Oil

2.10.1. Antifungal Activity

The antifungal activity of the different essential oils was tested against two wheat phytopathogenic fungi by direct contact, namely *Fusarium culmorum* (in Petri dish and adapted) [43,44] and *Zymoseptoria tritici* (in liquid medium), according to the FRAC protocol (Fungicide Resistance Action Committee) [45].

Briefly, 0.5 cm mycelial discs of *F. culmorum* were collected from a 7-day-old fungal colony and placed in the center of a 55 mm petri dish containing sterile potato dextrose agar (PDA) (40 g/L) as the culture medium. A concentration range of HEs was performed from 0.0005% to 0.08%, which was then incorporated into the culture medium at 50 °C. Analyses were conducted in triplicates. Samples were incubated for 4 days at 20 °C, and then the growth diameter of the mycelium was measured. The inhibition rate (IR) was calculated according to the following formula:

$$\text{IR (\%)} = (X_0 - X_i) / X_0 \times 100$$

where X_0 = average diameter of the fungal colony in control and X_i = average diameter of the fungal colony in the treatment.

Aqueous solutions of 1% (v/v) ethanol or HP- β -CD (10 mM) were tested as negative controls.

Graphical interpolation analysis was used to calculate the semi-maximal inhibitory concentration (IC₅₀) value.

For the test against *Z. tritici*, the essential oil tested was incorporated directly into the wells containing glucose–peptone. Each line of 12 wells representing a product concentration had four control wells for the stained character of the essential oil without fungal spore and 8 wells that constituted replicates with the fungus.

The fungus suspension was carried out with a 4-day-old inoculum for the *Z. tritici* strain. The spore suspension was calibrated by Malassez cell counting to contain 2×10^5 spores/mL according to a FRAC protocol [46]. We added an amount of 60 μ L per well of spores. The microplates were incubated in a culture chamber at 20 °C in the dark, under mechanical agitation (110 rpm) for 6 days.

After the time corresponds to an optimal development time for the fungus, the microplates were analyzed using a spectrometer. OD values were read at 620 nm without shaking by the spectrometer and analyzed manually.

For each product concentration, the net OD was calculated according to the following formula:

$$\text{Net OD} = \text{average OD with fungus} - \text{average OD control without spores}$$

Graphical interpolation analysis was used to calculate the semi-maximal IC₅₀ value.

2.10.2. Anti-Germinative Activity

The anti-germinative activity of essential oil by direct contact against *Blumeria graminis* f.sp conidia, the causal agent of wheat powdery mildew, was evaluated in vitro. A range of concentrations of essential oil (0.01% to 0.05%) of the three studied species was prepared and introduced in a sterile medium of Agar (15 g/L)–DMSO (1%), cooled at 50 °C. The spores of *B. graminis* were dispersed on the plates and then observed after 24 to 48 h under an optical microscope (Nikon Eslipse E600). The spores were counted and divided into two classes: ungerminated and germinated.

Graphical interpolation analysis was used to calculate the semi-maximal IC₅₀ value.

2.11. Statistical Analysis

Statistical analysis was performed by XLSTAT 2022.1.1 (Adinosoft), and statistical significance was analyzed using ANOVA supplemented with a Tukey HSD and Fisher (LSD) test.

The IC₅₀ was obtained by non-linear regression analyses from three replicates for the antifungal and phytotoxicity tests. The statistical significance of the results was evaluated by ANOVA supplemented by a Tukey HSD and Fisher test (LSD).

A statistical principal component analysis (PCA) based on Pearson's correlation matrix was performed with XLSTAT to identify possible correlations between the biological activities studied and growth parameters, primary metabolites, essential oil yields, and other parameters. Another PCA analysis was performed to study the correlation between the components of each essential oil and the biological activities, taking into account the compounds that differed between the essential oil of the inoculated plants and those of the non-inoculated ones.

3. Results

3.1. Mycorrhizal Colonization

Microscopic observations of stained roots revealed the presence of specific arbuscular mycorrhizal structures, hyphae, arbuscules, and vesicles in all AMF-inoculated thyme and lavender plants. No AMF structures were observed in the root of the non-inoculated AMP species.

The intensity of mycorrhizal colonization varied significantly ($p < 0.0001$) according to the AMF and AMP species. The highest colonization intensity (77.6%) was exhibited by *R. irregularis* associated with *L. dentata*, followed by *T. satureioides* inoculated with *R. irregularis* and *T. pallidus* colonized by *R. irregularis* (72.6 and 70%, respectively) (Figure 1). The intensity of mycorrhizal colonization exceeded 62.7, 64, and 59.3% in *T. satureioides*, *T. pallidus*, and *L. dentata*, respectively.

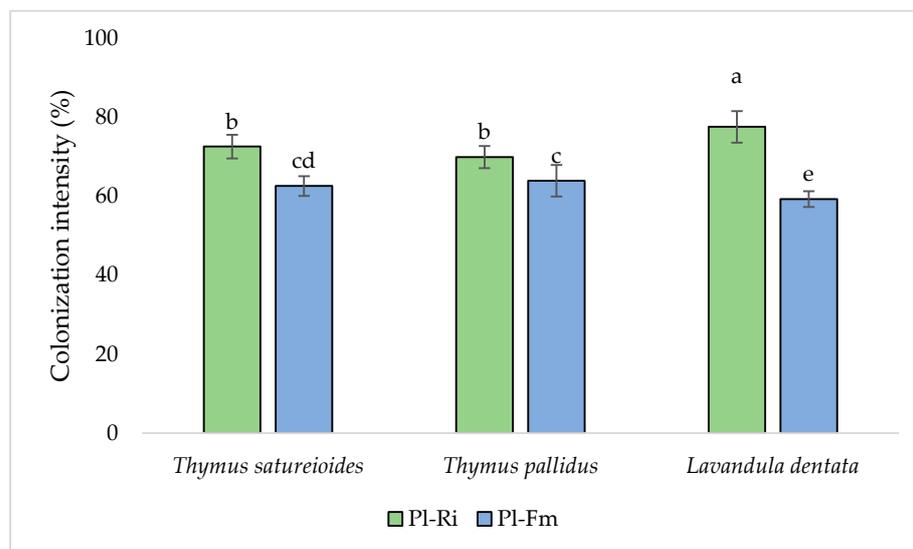


Figure 1. Intensity of mycorrhizal colonization (%) in roots of *Thymus satureioides* (Ts), *Thymus pallidus* (Tp), and *Lavandula dentata* (Ld) inoculated with *Rhizophagus irregularis* (Ri) or *Funneliformis mosseae* (Fm) after 4 months of culture under greenhouse conditions. Bars followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

3.2. Shoot Biomass

The biomass production expressed as shoot dry matter (SDM) per plant was significantly higher ($p < 0.0001$) in inoculated AMP (NI-AMP) than in non-inoculated ones (NI-AMP), with an improvement rate of up to 37.1, 52.42, and 43.6% in *T. satureioides*, *T. pallidus*, and *L. dentata* respectively. Indeed, the SDM was higher than 2.7, 2.6, and 1.9 g/plant in mycorrhizal plants and lower than 2.2, 2, and 1.3 g/plant in non-inoculated plants of *T. satureioides*, *T. pallidus*, and *L. dentata*, respectively (Figure 2). *F. mosseae* induced the highest biomass production when associated with *T. satureioides* (3.1 g/plant) and

T. pallidus (3.4 g/plant), while in *L. dentata*, SDM did not significantly ($p > 0.05$) differ between AMF strains (Figure 2).

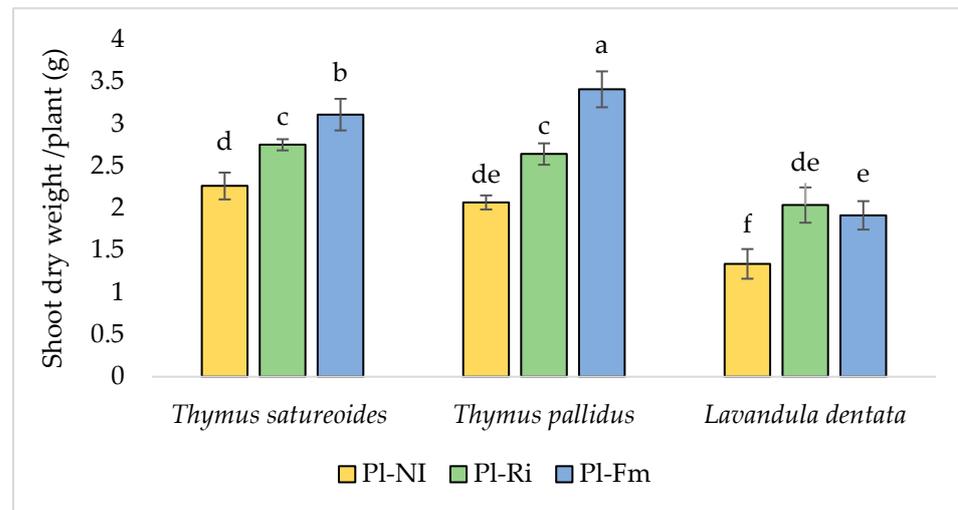


Figure 2. Shoot dry matter (SDM) per plant of *Thymus satureioides* (Ts), *Thymus pallidus* (Tp), and *Lavandula dentata* (Ld), non-inoculated (NI) or inoculated with *Rhizophagus irregularis* (Ri) or *Funneliformis mosseae* (Fm) after 4 months of culture under greenhouse conditions. Bars followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

3.3. Total Chlorophyll, Soluble Sugar, and Protein Content

Total chlorophyll content (TCC) varied significantly ($p < 0.0001$) according to the mycorrhizal status and AMP species. Compared to NI-AMP, TCC increased by 38.5, 81.5, and 51.4% in inoculated *T. satureioides*, *T. pallidus*, and *L. dentata*, respectively (Figure 3). Indeed, the total chlorophyll content expressed in mg/g of dry matter (DM) was higher than 2.2, 2.4, and 2 mg/g DM in inoculated plants (I-AMP) and did not exceed 1.6, 1.3, and 1.4 mg/g DM in non-inoculated plants (NI-AMP) of *T. satureioides*, *T. pallidus*, and *L. dentata*, respectively. No significant difference was observed between the total chlorophyll content recorded in Tp-Ri and Tp-Fm plants. However, the highest chlorophyll content (3 mg/g DM) was induced by *F. mosseae* in *T. satureioides* and by *R. irregularis* in *L. dentata* (Figure 3).

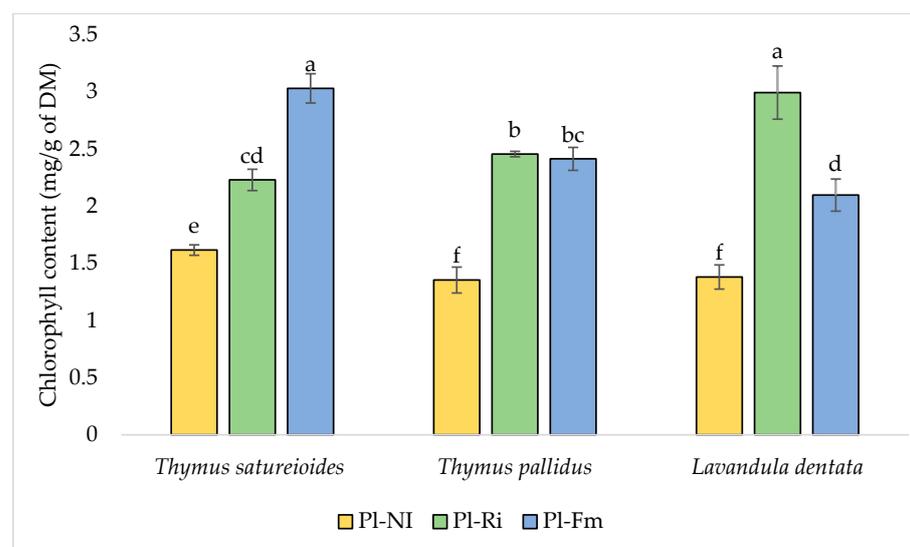


Figure 3. Chlorophyll content (mg/g DM) in *Thymus satureioides* (Ts), *Thymus pallidus* (Tp), and *Lavandula dentata* (Ld), non-inoculated (NI) or inoculated with *Rhizophagus irregularis* (Ri) or *Funneliformis mosseae* (Fm) after 4 months of culture under greenhouse conditions. Bars followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

The total soluble sugar content significantly increased ($p < 0.0001$) in I-AMP compared to their respective NI-AMP, regardless of the AMF strain or AMP species used. It varied between 62.2 and 185, 108.7 and 141.9, and 156 and 163.9 mg/g DM in mycorrhizal plants of *T. satureioides*, *T. pallidus*, and *L. dentata*, respectively, and did not exceed 30.7, 48.3, and 99.8 mg/g DM in their corresponding NI-AMP. The total soluble sugar content was 6 times higher in Ts-Fm, 2.2 times higher in Tp-Ri, and 1.5 times higher in Ld-Ri and Ld-Fm compared to NI-AMP (Figure 4).

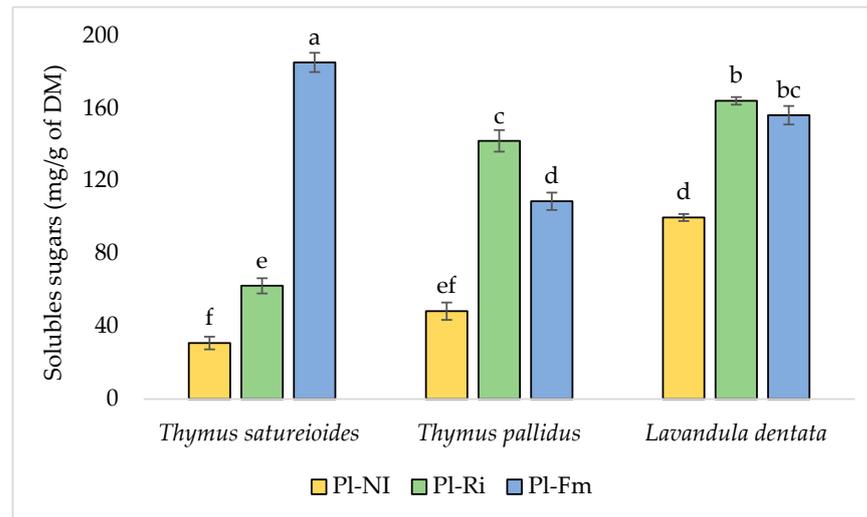


Figure 4. Soluble sugar content (mg/g DM) in *Thymus satureioides* (Ts), *Thymus pallidus* (Tp), and *Lavandula dentata* (Ld), non-inoculated (NI) or inoculated with *Rhizophagus irregularis* (Ri) or *Funneliformis mosseae* (Fm) after 4 months of culture under greenhouse conditions. Bars followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

The protein content varied significantly ($p < 0.0001$) depending on the AMF and AMP species. The total protein content in the three AMP species was more than 1.5 times higher in I-AMP than in NI-AMP (Figure 5).

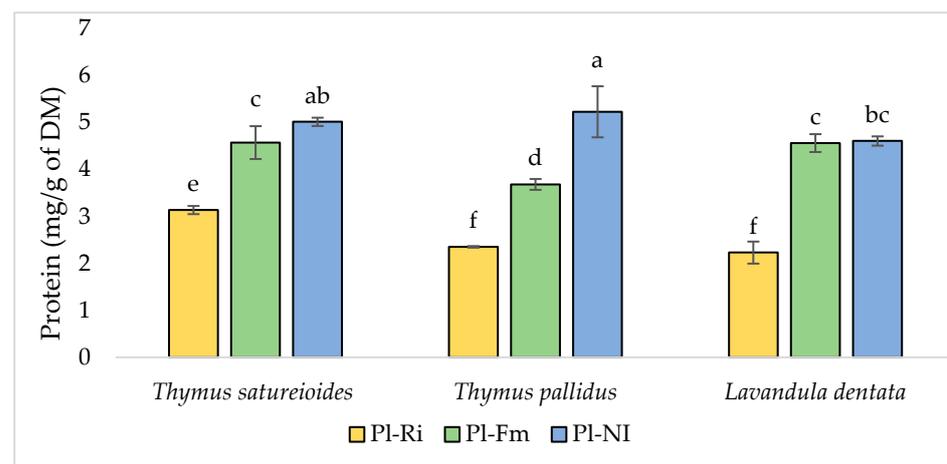


Figure 5. Protein content (mg/g of DW) in *Thymus satureioides* (Ts), *Thymus pallidus* (Tp), and *Lavandula dentata* (Ld), non-inoculated (NI) or inoculated with *Rhizophagus irregularis* (Ri) or *Funneliformis mosseae* (Fm) after 4 months of culture under greenhouse conditions. Bars followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

Protein content was highest in *T. satureioides* (5 mg/g DM) and *T. pallidus* (5.2 mg/g DM) associated with *F. mosseae*, but did not significantly differ between the two AMF strains in *L. dentata* (Figure 5).

3.4. Nitrogen, Phosphorus, and Potassium Content

Mycorrhizal inoculation significantly ($p < 0.0001$) increased the nitrogen content in both AMP species (Figure 6). Indeed, the nitrogen content did not exceed 0.6%, 0.7%, and 0.5% in NI-AMP of *T. satureioides*, *T. pallidus*, and *L. dentata*, respectively, while it was higher than 0.9, 1, and 0.9% in their respective I-AMP. The highest nitrogen content (1.25%) was recorded in *T. satureioides* associated with *F. mosseae* (Ts-Fm). However, no significant difference was observed between Tp-Ri and Tp-Fm, nor between Ld-Ri and Ld-Fm (Figure 6).

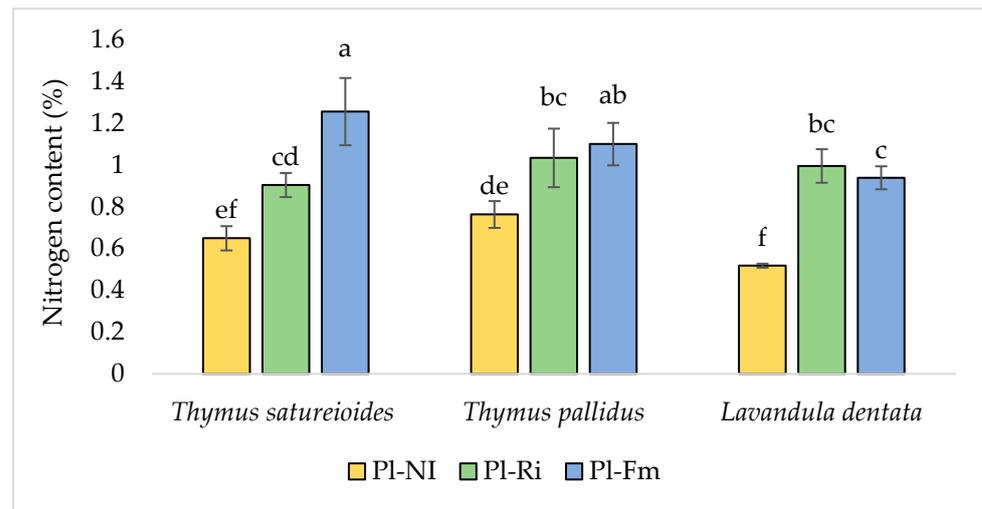


Figure 6. Nitrogen content (%) in *Thymus satureioides* (Ts), *Thymus pallidus* (Tp), and *Lavandula dentata* (Ld), non-inoculated (NI) or inoculated with *Rhizophagus irregularis* (Ri) or *Funneliformis mosseae* (Fm) after 4 months of culture under greenhouse conditions. Bars followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

Mycorrhizal inoculation significantly ($p < 0.0001$) increased the phosphorus and potassium content in both AMP species (Figure 7).

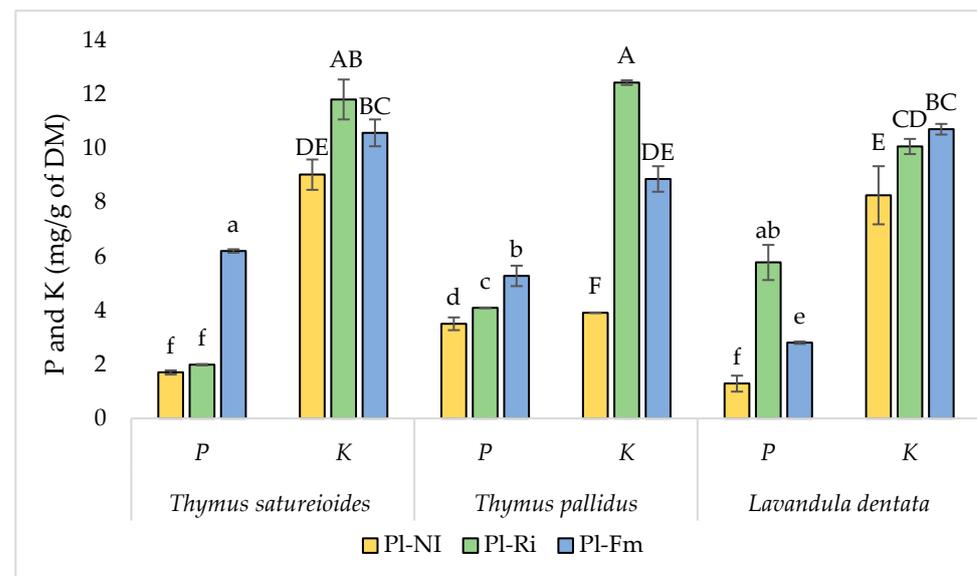


Figure 7. Phosphorus (P) and potassium (K) content (%) in *Thymus satureioides* (Ts), *Thymus pallidus* (Tp), and *Lavandula dentata* (Ld), non-inoculated (NI) or inoculated with *Rhizophagus irregularis* (Ri) or *Funneliformis mosseae* (Fm) after 4 months of culture under greenhouse conditions. Bars followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

Indeed, the phosphorus content did not exceed 1.7, 3.5, and 1.3 mg/g DM in NI-AMP of *T. satureioides*, *T. pallidus*, and *L. dentata*, respectively, while it was higher than 2, 4, and 2.8% in their respective I-AMP. Phosphorus content was highest in *T. satureioides* (6.2 mg/g DM) and *T. pallidus* (5.27 mg/g DM) associated with *F. mosseae*, while it was highest in *L. dentata* (5.7 mg/g DM) associated with *R. irregularis*.

The potassium content was higher than 10.5, 8.8, and 10 mg/g DM in I-AMP, while it did not exceed 9, 3.5, and 8.2 mg/g DM in NI-AMP of *T. satureioides*, *T. pallidus*, and *L. dentata*, respectively. The highest potassium content (12.4 mg/g DM) was recorded in *T. pallidus* associated with *R. irregularis* (Tp-Ri). However, no significant difference was observed between Ts-Ri and Ts-Fm, nor between Ld-Ri and Ld-Fm (Figure 7).

3.5. Essential Oil Yields

The essential oil yield was significantly ($p < 0.0001$) increased by AMF inoculation in the three AMP species (Figure 8). Essential oil production was higher than 8.5, 5, and 3.3 $\mu\text{L/g}$ dry matter (DM) in I-AMP, while it did not exceed 7.16, 3.8, and 2.5 $\mu\text{L/g}$ DM in NI-AMP of *T. satureioides*, *T. pallidus*, and *L. dentata*, respectively.

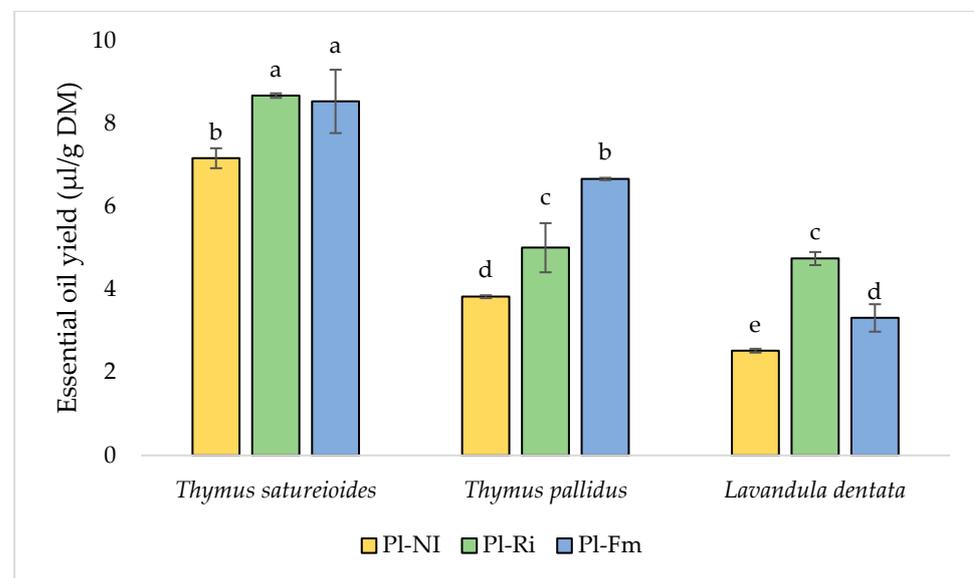


Figure 8. Essential oil yield distilled from *Thymus satureioides* (Ts), *Thymus pallidus* (Tp), and *Lavandula dentata* (Ld), non-inoculated (NI) or inoculated with *Rhizophagus irregularis* (Ri) or *Funnelformis mosseae* (Fm) after 4 months of culture under greenhouse conditions. Bars followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

The essential oil production varied significantly between the AMF species in *T. pallidus* and *L. dentata*; *F. mosseae* induced higher essential oil yield (6.6 $\mu\text{L/g}$ DM) than *R. irregularis* (5 $\mu\text{L/g}$ DM) in *T. pallidus*. Meanwhile, in lavender, *R. irregularis* produced the highest (4.7 $\mu\text{L/g}$ DM) essential oil yield (Figure 8). In *T. satureioides*, essential oil yield was increased by 10% in mycorrhizal plants compared to non-inoculated ones, but did not significantly differ between Ts-Ri and Ts-Fm.

3.6. Essential Oil Chemical Composition

The analysis of the essential oil composition revealed that mycorrhizal inoculation did not affect the nature of the essential oil compounds, but it affected the abundance of some major compounds such as oxygenated monoterpenes in *T. satureioides* and *L. dentata*. This mycorrhizal effect varied depending on AMF species.

More than 21 compounds were identified in the essential oil of *T. satureioides*. The abundance of oxygenated monoterpenes was more important in inoculated plants than in non-inoculated ones. It varied between 58.32% in Ts-Ri and 86.65% in Ts-Fm, while it

did not exceed 57.96% in non-inoculated plants of *T. satureioides* (Table 1). The abundance of hydrocarbon monoterpenes such as tricyclene, α -pinene, camphene, β -pinene, and γ -terpinene decreased from 2.1, 2.06, 0.65, 0.47, and 4.18% in non-inoculated plants to 1.19, 0.06, 0.46, 0.28, and 2.04% in mycorrhizal ones, respectively. These reductions were compensated by the abundance of other compounds such as carvacrol methyl ether, thymol, and carvacrol, which increased from 11.28, 16.55, and 18.5% in non-inoculated plants to 15.44, 23.77, and 23.36% in I-AMP, respectively. Indeed, this increase of borneol was more pronounced, and it was three times higher in inoculated *T. satureioides* (16.73% in Ts-Ri and 18.68% in Ts-Fm) compared to the non-inoculated one (5.93%) (Table 1).

Table 1. Chemical composition of essential oil (in %) in inoculated and non-inoculated *T. satureioides* after 4 months of culture under greenhouse conditions. RT—retention time; IK—Retention indices calculated relative to n-alkanes (C-8 to C-40) using capillary column ZB-5MS; IK*—Retention indices found in the literature and The Pherobase [47]: Database of Pheromones and Semiochemicals; Ts-NI—*Thymus satureioides*, non-inoculated; Ts-Ri: *Thymus satureioides* inoculated with *R. irregularis*; Ts-Fm—*Thymus satureioides* inoculated with *F. mosseae*.

Name	RT	IK	IK*	Ts-NI	Ts-Ri	Ts-Fm
Tricyclene	1.26	919	919	2.1	1.19	-
α -pinene	1.34	937	933	2.06	1.27	0.06
Camphene	1.49	967	952	0.65	0.46	-
β -Pinene	1.54	979	981	0.47	0.28	-
α -terpinene	1.74	1018	1018	8.47	8.37	3.11
δ -Terpinene	1.93	1053	1059	4.18	3.4	2.04
cis-sabinene hydrate	2.01	1066	1069	0.14	0.07	0.07
Linalool	2.17	1097	1098	1.67	1.62	1.84
Borneol	2.61	1173	1165	5.93	16.73	18.68
Terpinen-4-ol	2.65	1181	1179	0.97	1.1	1.09
Cis dihydrocarvone	2.74	1196	1198	2.71	2.29	2.23
Carvacrol methylether	2.98	1239	1244	11.28	13.47	15.44
Bornyl acetate	3.23	1284	1285	0.21	0.24	0.17
Thymol	3.27	1292	1290	16.55	16.75	23.77
Carvacrol	3.33	1301	1299	18.5	6.05	23.36
α -Copaene	3.73	1377	1376	0.13	0.18	0.17
β - Caryophyllene	3.97	1424	1428	2.26	2.57	2.99
α - Humulene	4.15	1458	1460	0.34	0.41	0.54
δ -Muurolene	4.44	1515		0.15	0.17	0.21
δ -Cadinene	4.46	1519		0.34	0.42	0.51
Caryophyllene oxide	4.77	1585	1573	0.25	0.28	0.43
Tau-Cadinol	5.05	1644	1640	0.34	0.36	0.46
Oxygenated monoterpene				57.96	58.32	86.65
Hydrocarbon monoterpene				17.93	14.97	5.21
Oxygenated sesquiterpene				0.59	0.64	0.89
Hydrocarbon sesquiterpene				3.22	3.75	4.42

For the essential oil of *T. pallidus*, 16 compounds were identified, showing a greater abundance of hydrocarbon monoterpenes in inoculated plants than in non-inoculated plants. It varied from 34.6% in Tp-Ri to 42.9% in Tp-Fm, while it did not exceed 25.8% in non-inoculated *T. pallidus*. Compared to Tp-NI, mycorrhizal inoculation induced (1) a decrease in some compounds, such as thymol, which decreased by 18.84% in Tp-Ri and 22.48% in Tp-Fm, and borneol, which decreased by 6.33% in Tp-Ri and 62.53% in Tp-Fm, and (2) increased abundances of some compounds (α -terpinene, caryophyllene oxide, camphene, and verbenene), which were two times higher in the essential oil of I-AMP with respect to in non-inoculated plants (Table 2).

Table 2. Chemical composition of essential oil (in %) in inoculated and non-inoculated *T. pallidus* after 4 months of culture. RT—retention time; IK—Retention indices calculated relative to n-alkanes (C-8 to C-40) using capillary column ZB-5MS; IK*—Retention indices found in the literature and The Pherobase [47]: Database of Pheromones and Semiochemicals; Tp-NI—*Thymus pallidus*, non-inoculated; Tp-Ri—*Thymus pallidus* inoculated with *R. irregularis*; Tp-Fm—*Thymus pallidus* inoculated with *F. mosseae*.

Nom	RT	IK	IK*	Tp-NI	Tp-RI	Tp-Fm
Tricyclene	1.26	919	919	0.09	1.46	0.58
Camphene	1.34	936	952	0.04	1.44	0.88
Verbenene	1.49	967	967	0.02	0.37	0.18
(+)-4-Carene	1.71	1010	1011	0.01	0.49	0.62
α -terpinene	1.76	1020	1018	18.63	22.3	32.6
δ -Terpinene	1.93	1053	1059	7.05	8.6	8.12
Linalool	2.18	1097	1098	6.35	5.15	7.39
Borneol	2.61	1172	1165	16.09	15.07	6.03
Terpinen-4-ol	2.65	1180	1178	0.79	0.98	0.87
Isobornyl acetate	3.24	1285	1285	0.1	0.08	0.02
Thymol	3.29	1294	1290	37.14	30.14	28.79
Carvacrol	3.32	1300	1298	6.88	7.74	4.86
Caryophyllene	3.97	1423	1418	2.57	2.16	3.64
Humulene	4.15	1458	1460	0.18	0.16	0.09
Caryophyllene oxide	4.78	1584	1581	0.78	0.5	0.8
α -cadinol	5.05	1643	1652	0.02	0.09	-
Oxygenated monoterpene				67.35	59.16	47.96
Hydrocarbon monoterpene				25.84	34.66	42.98
Oxygenated sesquiterpene				0.78	0.59	0.8
Hydrocarbon sesquiterpene				2.75	2.32	3.73

More than 24 compounds were identified in the essential oil of *L. dentata*, revealing an increased abundance of oxygenated monoterpenes in mycorrhizal plants (exceeding 63% in Ld-Ri and 75.29% in Ld-Fm) compared to non-inoculated plants (58.23%). Compounds were slightly modified when comparing inoculated and non-inoculated plants, and between Ld-Ri and Ld-Fm (Table 3). The main compound in the essential oil of Ld-NI and Ld-Ri was isoborneol, and camphor in Ld-Fm. Moreover, increased abundances of phenol, camphor, and isobornyl were noticed in I-AMP, accompanied by a decrease in minor compounds such as Magdalene.

3.7. Biological Activities of Essential Oil

3.7.1. Anti-Germinative Activity

The anti-germinative activity of the essential oil against *B. graminis* varied significantly ($p < 0.0001$) according to the mycorrhizal status and the AMP species (Figure 9). Indeed, the IC_{50} was lower than 8.2, 17.1, and 17.3 $\mu\text{g/mL}$ of essential oil in inoculated plants, while it exceeded 13, 26, and 27 $\mu\text{g/mL}$ of essential oil in non-inoculated plants of *T. saturoioides*, *T. pallidus*, and *L. dentata*, respectively. However, the essential oil's anti-germinative activity did not significantly vary among AMF strains, regardless of AMP species.

Table 3. Chemical composition of essential oil (in %) in inoculated and non-inoculated *L. dentata* after 4 months of culture. RT—retention time; IK—Retention indices measured relative to n-alkanes (C-8 to C-40) using capillary column ZB-5MS; IK*—Retention indices found in the literature and The Pherobase [47]: Database of Pheromones and Semiochemicals; Ld-NI—*Lavandula dentata*, non-inoculated; Ld-Ri—*Lavandula dentata* inoculated with *Rhizophagus irregularis*; Ld-Fm—*Lavandula dentata* inoculated with *F. mosseae*.

Name	RT	IK	IK*	Ld-NI	Ld-Ri	Ld-Fm	
Tricyclene	1.26	918	919	-	1.25	0.88	
α - pinene	1.34	936	937	-	1.04	1.46	
Camphene	1.49	966	953	0.54	2.89	1.89	
D-limonene	1.77	1022	1031	-	1.61	0.69	
Eucalyptol	1.79	1025	1033	-	-	0.79	
L-fenchone	2.1	1083	1062	1.78	3.19	1.19	
Linalool	2.18	1097	1098	-	-	0.68	
Fenchol	2.31	1121	1123	17.84	19.5	4.14	
α -campholenal	2.34	1125	1127	-	0.85	-	
(1r)-(+)-nopiune	2.43	1141	1142	1.36	1.17	-	
Camphor	2.47	1148	1143	6.89	8.19	58.44	
Pinocarvone	2.55	1162	1162	0.8	0.91	0.72	
Isoborneol	2.64	1177	1177	25.12	27.04	8.87	
Terpinene-4-ol	2.7	1189	1179	0.51	-	-	
Myrtenal	2.74	1195	1195	2.61	2.7	1.8	
Carveol	2.92	1228	1229	2.21	3.06	0.64	
Cuminaldehyde	3.01	1244	1239	0.89	-	-	
Bornyl acetat	3.24	1284	1285	0.43	0.51	0.28	
Germacrene D	4.32	1490	1499	4.11	6.76	3.65	
Geranyl-, α -terpinene	4.71	1570	-	-	-	0.83	
Caryophyllene oxide	4.78	1586	1581	3.61	4.3	1.92	
Valencen	5.01	1634	-	0.42	0.3	-	
β -eudesmol	5.12	1657	1654	7.98	10.37	6.05	
Cadalene	5.19	1673	1674	0.62	0.39	-	
				Oxygenated monoterpene	58.23	63.42	75.29
				Hydrocarbon monoterpene	3.11	10.46	8.04
				Oxygenated sesquiterpene	14.04	17.48	11.41
				Hydrocarbon sesquiterpene	5.15	7.45	3.65

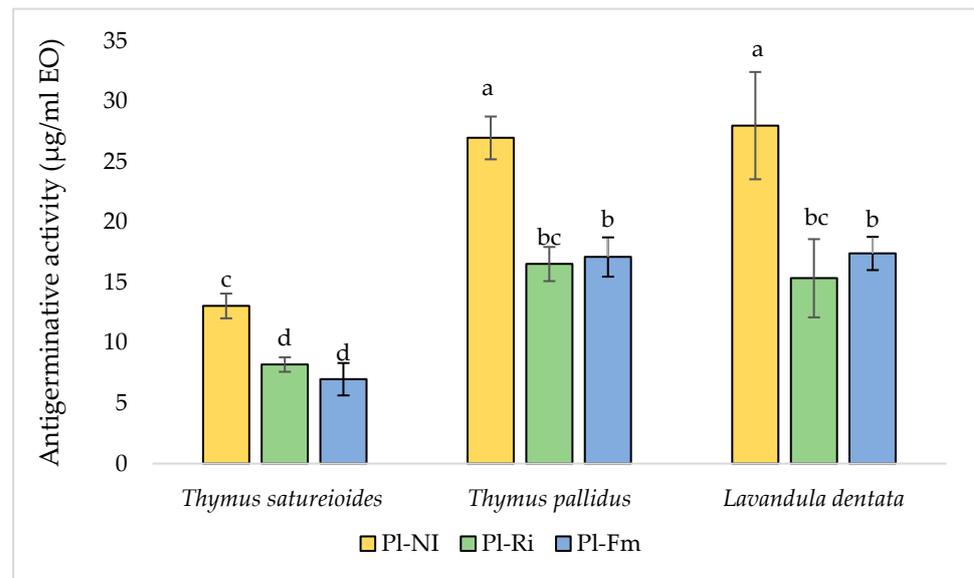


Figure 9. Anti-germinative activity of the essential oil of *Thymus satureioides* (Ts), *Thymus pallidus* (Tp), and *Lavandula dentata* (Ld), non-inoculated (NI) or inoculated with *Rhizophagus irregularis* (Ri) or *Funneliformis mosseae* (Fm) after 4 months of culture under greenhouse conditions Bars followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

3.7.2. Antifungal Activities

The antifungal activity of the essential oil against *Z. tritici* varied significantly according to the AMF and AMP species (Figure 10). The IC_{50} lower than 4.1 µg/mL, 4.7 µg/mL, and 1 mg/mL of essential oil in the inoculated plants of *T. satureioides*, *T. pallidus*, and *L. dentata*, respectively. In comparison, it was higher than 6 µg/mL, 11 µg/mL, and 3 mg/mL of essential oil in their respective non-inoculated plants. There was no significant difference in the antifungal activity of the essential oil against *Z. tritici* between the two AMF species in *T. pallidus*, whereas *R. irregularis* induced the highest antifungal activity against *Z. tritici* when associated with *T. satureioides* or with *L. dentata*.

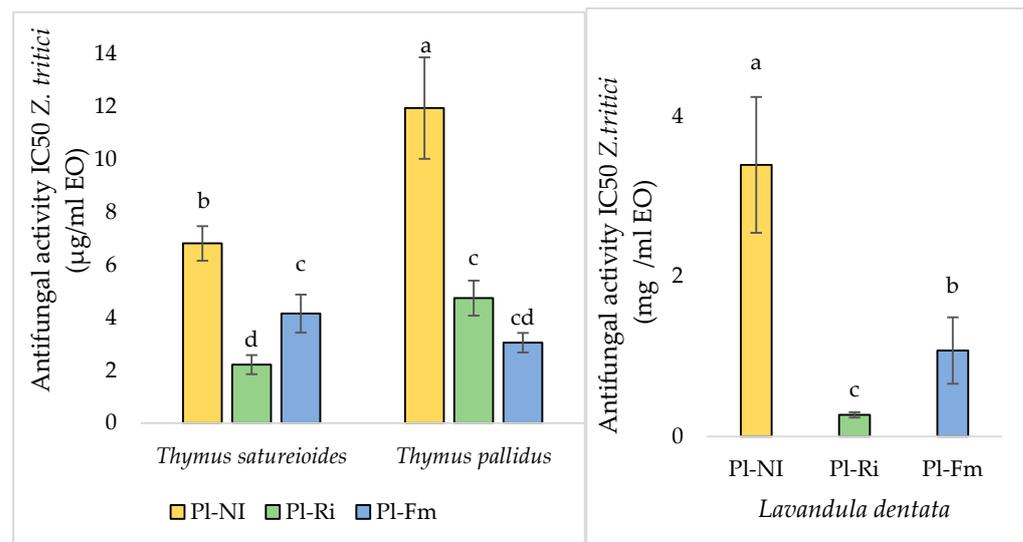


Figure 10. Antifungal activity IC_{50} of essential oil of *Thymus satureioides* (Ts), *Thymus pallidus* (Tp), and *Lavandula dentata* (Ld), non-inoculated (NI) or inoculated with *Rhizophagus irregularis* (Ri) or *Funneliformis mosseae* (Fm) after 4 months of culture under greenhouse conditions Bars followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

Concerning the antifungal activity against *Fusarium culmorum*, the IC₅₀ was lower than 146.8, 217.9, and 363.8 µg/mL of essential oil in inoculated plants but higher than 174, 327, and 494 µg/mL of essential oil in non-inoculated plants of *T. satureioides*, *T. pallidus* and *L. dentata*, respectively (Figure 11). However, the antifungal activity of the essential oil against *F. culmorum* did not significantly differ between Ld-Ri and Ld-Fm. At the same time, the lowest IC₅₀ was recorded in *T. satureioides* associated with *R. irregularis* (103.76 µg/mL of essential oil) and *T. pallidus* associated with *F. mosseae* (146.8 µg/mL of essential oil).

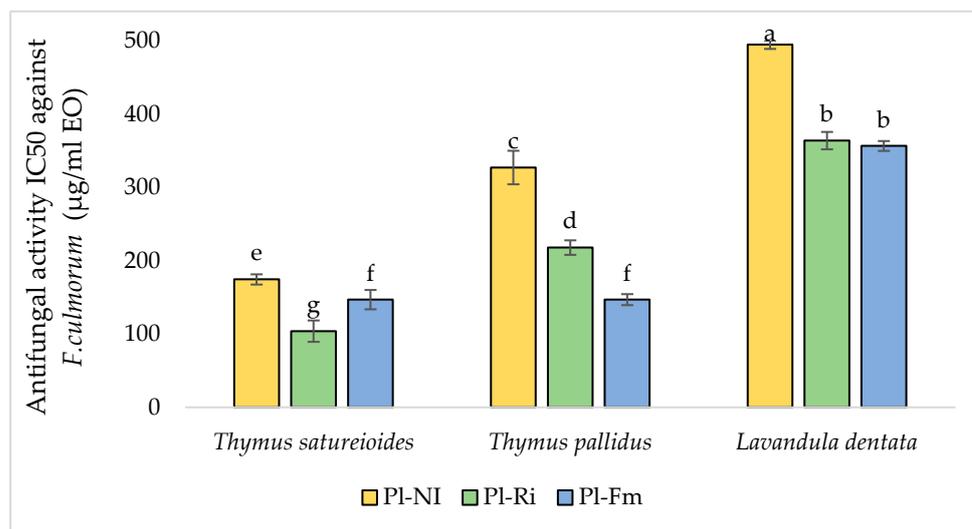


Figure 11. Antifungal activity IC₅₀ of essential oil of *Thymus satureioides* (Ts), *Thymus pallidus* (Tp), and *Lavandula dentata* (Ld), inoculated with *Rhizophagus irregularis* (Ri) or *Funneliformis mosseae* (Fm) after 4 months of culture under greenhouse conditions. Bars followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

4. Discussion

The present study aimed at investigating the potential role of two AMF species (*R. irregularis*, and *F. mosseae*) in improving biomass and essential oil production in three AMP with important economic value which are unfortunately under threat of extinction (*T. satureioides*, *T. pallidus*, and *L. dentata*).

Our results show the occurrence of functional mycorrhiza in these AMP species regardless of the AMF used. Indeed, mycorrhizal intensities exceeded 59% in both inoculated lavender and thyme plants. Moreover, the present study confirms that AMP exist within plant species with mycorrhizal dependency. The beneficial effect of AMF on AMP growth has been reported in several other aromatic plant species, such as *Salvia officinalis* L., *Acacia gummifera*, and *Ocimum basilicum* L. [27,48,49], as exhibited by increases in biomass production (1.5 times higher) and nitrogen, phosphorus, and potassium content (1.1 to 2 times higher) in AMF-inoculated AMP compared to their respective non-inoculated plants. The high biomass production of mycorrhizal plants was positively correlated to the high acquisition of nitrogen, phosphorus, and potassium (Figures 6 and 7). These results are in agreement with previous reports on *Coriandrum sativum* L. inoculated with *R. irregularis*, which showed increased shoot concentration of nitrogen (44%), phosphorus (254%), and potassium (27%) [50]. It has been shown that the extra-matrical mycelium of AMF ramifies and grows into the surrounding soil, developing an extensive three-dimensional network of mycelia exploring the surrounding soil for mineral nutrients [51]. The length of the external hyphae growing in soil associated with mycorrhizal roots reaches an average of up to 10–14 m/cm root [21], increasing the root absorbing surface by 100 or even 1000 times [20,52–54]. This mycelial network can bridge over the zone of nutrient depletion around the roots to absorb low-mobility ions from the bulk soil.

Moreover, our study revealed a clear contribution of AMF in increasing the total chlorophyll, soluble sugars, and protein content by up to 2 times in inoculated thyme and

lavender plants compared to non-inoculated ones, in accordance with the research carried out by Yadav et al., which also showed an increase in those parameters in mycorrhizal *Gloriosa superba* L. [55]. Thus, a clear positive correlation (Figure 12) was noticed between the mycorrhizal colonization and the accumulation of chlorophyll, soluble sugar, and proteins. According to Alipour et al. [56], the increased leaf chlorophyll content in mycorrhizal plants is related to increased nutrient (N, P, K.) acquisition. Moreover, a positive linear correlation between leaf N concentration and leaf chlorophyll content was reported [57,58], thereby improving the photosynthetic potential of mycorrhizal plants [59]. Indeed, increasing the concentration of photosynthetic pigments (chlorophyll) by AMF improves the photosynthetic potential of their host plants, consequently improving their soluble sugar content [59,60]. The increased accumulation of chlorophyll, soluble sugars, and proteins in the mycorrhizal plant of thymes and lavender is another argument for their high photosynthetic potential [61,62], highlighting the relation between the synthesis pathway of these different metabolites and secondary metabolites (such as terpenoids) which are primary constituents of essential oils [63–65]. Indeed, in the current work, we showed that AMF inoculation also improved essential oil yield, which was 1.2, 1.7, and 1.9 times higher in *T. satureioides* (associated with *R. irregularis*), *T. pallidus* (associated with *F. mosseae*), and *L. dentata* (associated with *R. irregularis*), respectively, than in their respective non-inoculated plants. These findings are in line with several previous studies on inoculated AMP of *Atractylodes lancea*, *Artemisia umbelliformis*, and *Ocimum basilicum* L. [27,65]. Mycorrhizal AMP performance in terms of essential oil yield may be related to their nutritional and/or non-nutritional status, such as their level of endogenous phytohormones. Nutritional factors (sugar, proteins, N, P, etc.) are crucial in developing and multiplying glandular trichomes, essential oil channels, and secretion channels [66]. Indeed, the enhanced nutrient status of mycorrhizal plants enhanced the biosynthesis mechanisms of terpenoid precursors, including the mevalonate pathway (acetyl-CoA, ATP, and NADPH) as well as the methyl erythritol phosphate pathway (glyceraldehyde phosphate and pyruvate) [67]. Our result (Figure 12) shows a positive correlation between the primary and secondary metabolites (such as terpenes). Moreover, all precursors mentioned above result from primary metabolism [68]. In addition, AMF can change the phytohormone (jasmonic acid, gibberellic acid, 6-benzyl amino purine) concentrations, thereby increasing the number and size of glandular trichomes and enhancing the sesquiterpenoid biosynthetic mechanism [69]. The relationship between the increase in glandular trichome density, the increase in terpenoid concentration, and the yield of essential oil has been reported in many aromatic plants (e.g., *Mentha x Piperita*, *Phaseolus lunatus*, and *Lavandula angustifolia*) [70–72], confirming the observed positive correlation (Figure 12) between the colonization intensity and the essential oil yield.

Regarding the chemical composition of essential oil, no significant difference was detected between mycorrhizal and non-mycorrhizal plants. However, the abundance of the major compounds significantly varied according to AMF and AMP species. The abundance of oxygenated monoterpenes was higher in *T. satureioides* and *L. dentata* essential oils. However, in *T. pallidus* essential oil, there are hydrocarbon monoterpenes and oxygenated sesquiterpenes with a higher percentage. Concomitantly, hydrocarbon monoterpenes decreased in the essential oil of *T. satureioides* and *L. dentata*, while oxygenated monoterpenes decreased in the essential oil of *T. pallidus*. These results support the fact that the AMP studied are AMF dependent [73–76]. Indeed, the essential oil of *T. satureioides* inoculated with *F. mosseae* is characterized by a higher abundance of carvacrol, linalool, thymol, β -caryophyllene, carvacrol methyl ether, terpinene-4-ol, and borneol compared to the plant inoculated with *R. irregularis* (Figure 13A). However, in *T. pallidus*, association with *F. mosseae* exhibited a higher abundance of caryophyllene, α -terpinene, and (+)-4-carene, in addition to other compounds such as γ -terpinene, camphene, verbenene, and terpinene-4-ol compared to *T. pallidus* associated with *R. irregularis* (Figure 13B). On the other hand, *L. dentata* inoculated with *R. irregularis* synthesized more tricyclene, D-limonene, germacrene D, L-fenchone, β eudesmol, bornyl acetate, and carveol. In contrast, the essential

oil of lavender plants inoculated with *F. mosseae* was characterized principally by the high abundance of camphor (Figure 13C).

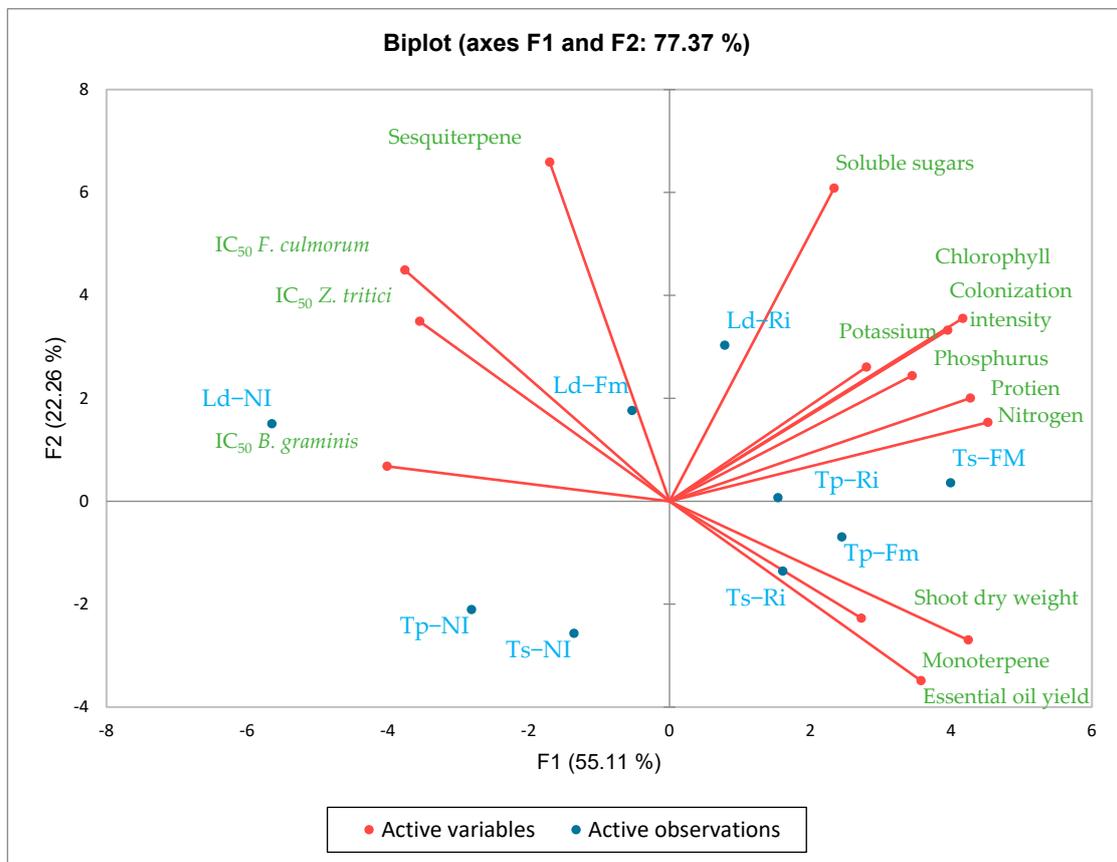


Figure 12. Correlation circles from the principal component statistical analyses (PCA) on different parameters studied for *Thymus satureioides* (Ts), *Thymus pallidus* (Tp), and *Lavandula dentata* (Ld), non-inoculated (NI) or inoculated with *Rhizophagus irregularis*, (Ri) or *Funneliformis mosseae*.

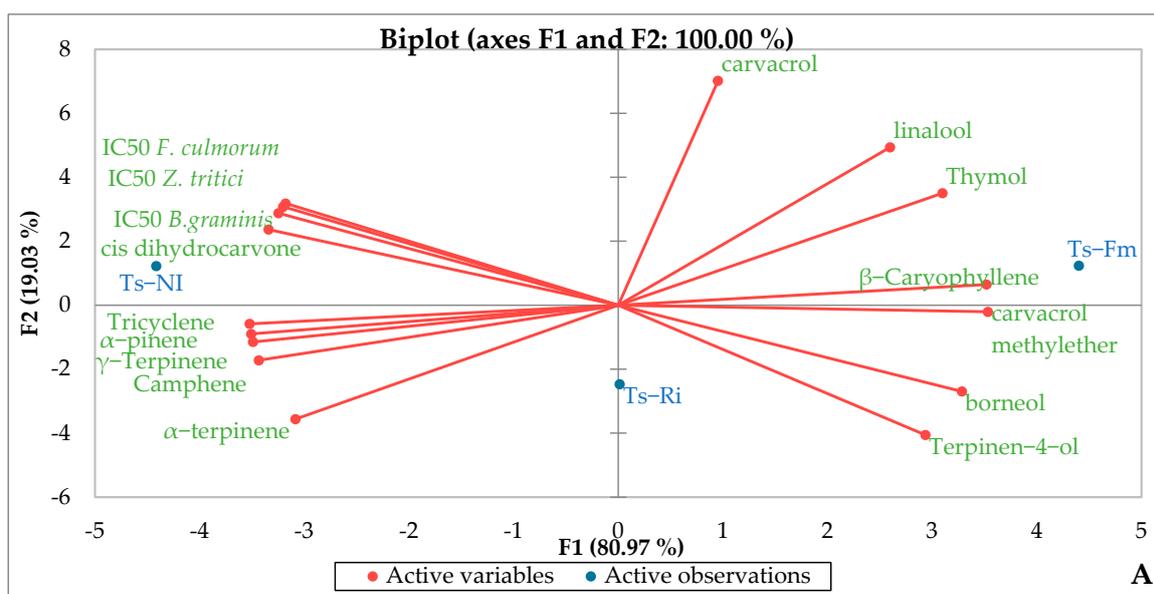


Figure 13. Cont.

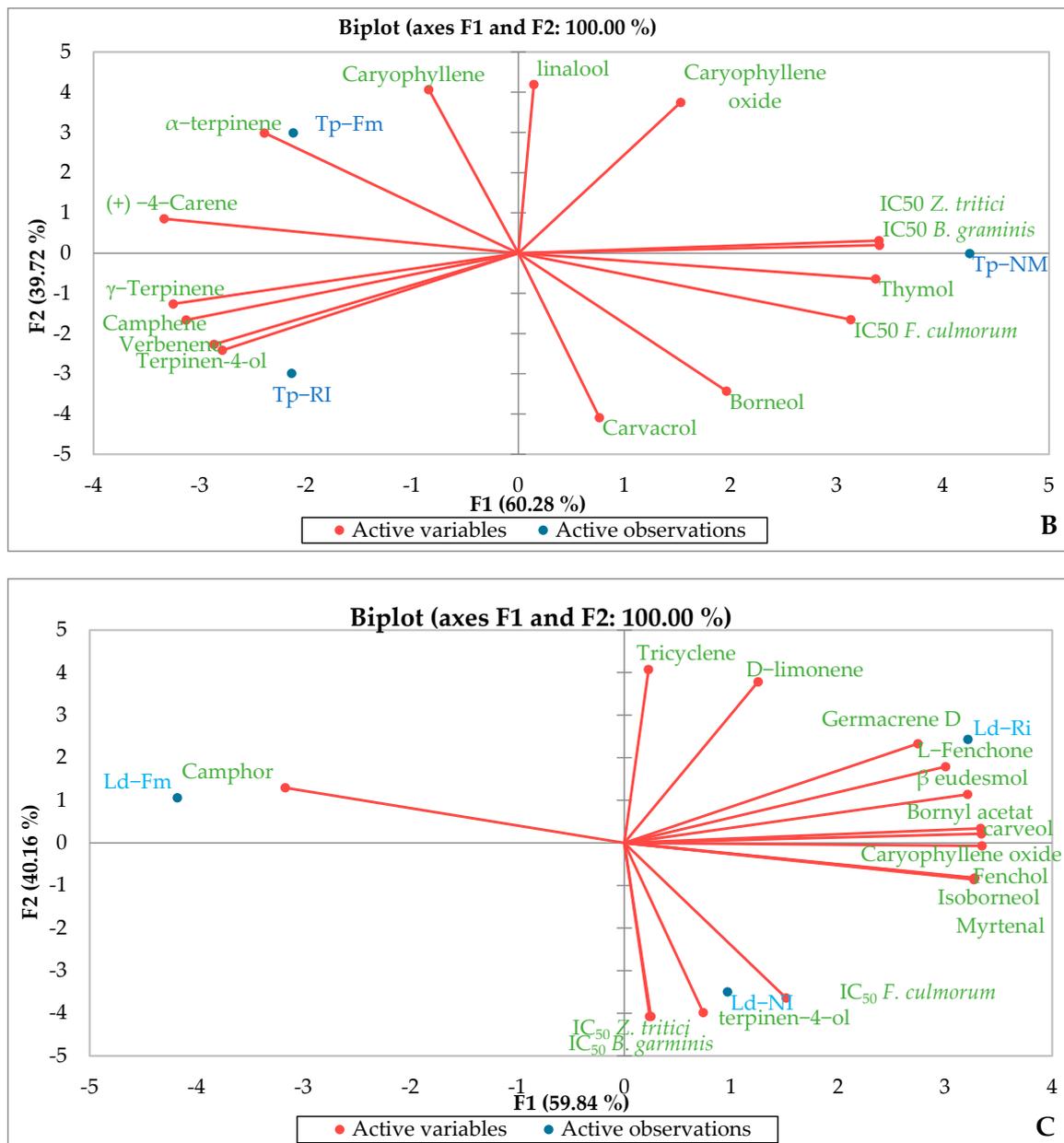


Figure 13. Correlation circles from the principal component statistical analyses (PCA) on antifungal activity and different chemical compounds in the essential oils of *Thymus satureioides* (Ts) (A), *Thymus pallidus* (Tp) (B), and *Lavandula dentata* (Ld) (C), non-inoculated (NI) or inoculated with *Rhizophagus irregularis*, (Ri) or *Funnelformis mosseae*.

The abundance of the mentioned compounds was negatively correlated to the IC₅₀ value of the antifungal activity, regardless of AMP species. Consequently, if these components are present in greater abundance in the essential oil, this will positively affect the activity of the essential oil. Indeed, the essential oil of mycorrhizal AMP showed higher antifungal activity against three wheat pathogenic fungi (*B. graminis*, *F. culmorum*, and *Z. tritici*) compared to the essential oil of non-inoculated AMP. Mycorrhizal plants' essential oil efficiency is mainly due to their higher monoterpene content [77–79]. This is confirmed by the negative correlation (Figure 12) between the abundance of monoterpenes and the IC₅₀ values. The contribution of AMF symbiosis to the host plant's performance in terms of essential oil effectiveness against phytopathogenic fungi results from a combination of physiological and metabolic effects [80]. It appears to be due to improved nutrient uptake and photosynthetic compound accumulation, leading to differences in nutritional status

between mycorrhizal and non-mycorrhizal plants. However, additional mechanisms have been proposed, including alteration of the structure and functions of the fungal cell membrane and the disturbance of DNA, RNA, and protein synthesis, in addition to the alteration of mitochondrial function and ATP production. The essential oil can alter ergosterol biosynthesis, which increases membrane fluidity and, consequently, ionic permeability, causing cellular death [80]. They can inhibit the β -glucan and chitin synthesis, alter the integrity of the fungal cell walls, disturb homeostasis, and lead to cell death [81–83]. The essential oils can compromise membrane functions such as nutrient transport, enzyme activity, and electron transport [84–86]. Then, they can affect mitochondrial ATPase, reducing ATP production and decreasing pH [87]; inhibit fungal cell wall formation, cell division and disturb RNA, DNA, and protein synthesis [80]. Oliveira Lima et al. [88] demonstrated the inhibition of conidia germination of *Trichophyton rubrum* by linalool, a monoterpene; this anti-germinative effect was related to the inhibition of germ tube formation, which is the first step of germination. According to Yamaoka et al. [89], this tube appears during the first two hours of the germinating process, proving that the inhibitory action of the essential oil occurs during the first two hours following inoculation.

5. Conclusions

Our results demonstrate that AMF could be used in the sustainable production of AMP that are endemic and/or endangered. Indeed, *T. satureioides*, *T. pallidus*, and *L. dentata* inoculated with *R. irregularis* and *F. mosseae* displayed higher biomass production than non-inoculated plants. This increase in plant biomass can be explained by an improvement in nutrient uptake (such as N, P, and K) caused by the AMF. The mycorrhizal AMP also contained higher levels of leaf chlorophyll, soluble sugars, and protein, indicating improved photosynthesis activity.

Moreover, the mycorrhizal AMP showed an increase in essential oil yield of more than 21%. While there were no differences in the chemical profile of EOs of the different AMP species studied, we detected an improvement in the abundance of oxygenated monoterpenes such as thymol (23.7%), carvacrol (23.3%), and borneol (18.7%) in *T. satureioides*; α -terpinene (32.6%), thymol (28.8%), and δ -terpinene (8.1%) in *T. pallidus*; and camphor (58.4%), isoborneol (8.8%), and fenchol (4.1%) in *L. dentata*. In addition, the biological activities were also positively affected by inoculation with AMF, regardless of the phytopathogenic fungi tested (*B. graminis*, *F. culmorum*, or *Z. tritici*). This improvement was correlated with an increase in the main compounds and their synergic effect.

Taken all together, our findings highlight the performance of *F. mosseae* in association with *Thymus*, while *R. irregularis* performs well in association with *Lavandula*.

Overall, AMF inoculation could constitute an alternative eco-technological approach to reduce chemical fertilizer application in the production of *T. satureioides*, *T. pallidus*, and *L. dentata* and alleviate the threat factors faced by endemic AMP.

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