Serbian Society of Soil Science
University of Belgrade, Faculty of Agriculture

# BOOK OF PROCEEDINGS

3rd International and 15th National Congress

## SOILS FOR FUTURE UNDER GLOBAL CHALLENGES


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# INFLUENCE OF MYCORRHIZAL FUNGI ON SATUREJA MONTANA L. GROWN IN LEACHED CHERNOZEM AND ARENOSOL

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## Abstract

*Satureja montana* L. is a valuable perennial medicinal and aromatic plant belonging to Lamiaceae family. In this paper, the effects of mycorrhizal fungi (mix of spores of *Glomus mosseae* and *G. intraradices*) were studied on growth of the aboveground part of *S. montana* potted plants. Winter savory plants were grown in low carbonate Chernozem with high clay content and typical Arenosol with low plant-available phosphorus in both soil types. It is known that mycorrhizal fungi can improve the phosphorus nutrition of plants under phosphorus limiting conditions, enhance plant growth, and increases the yield of crop plants. Prior to the pot experiment made in a field, the winter savory was vegetatively propagated by softwood cuttings. Rooted cuttings were transplanted into 1.5 L plastic pots (one plant per pot) filled with Chernozem and Arenosol taken from the plow layer of the soils in a disturbed condition. The experiment was set in a split-plot design with 4 replications. The main plots were soil types, while sub-plots were 2 treatments (inoculated and non-inoculated plants) with 6 pots in a random arrangement in each repetition. Plants were watered regularly with an installed drip irrigation system and weeds were regularly removed. At 60 days after inoculation with mycorrhizal fungi, non-inoculated and inoculated plants were harvested and the absolute stems and leaves dry masses of plants were recorded. Inoculated plants, in both soil types, had higher absolute stems and leave dry masses compared to absolute stems and leaves dry masses in non-inoculated plants. The absolute stems and leave dry masses in Chernozem and Arenosol of inoculated plants ranged from 0.37 to 1.16 g and from 0.35 to 0.73 g, respectively; while in non-inoculated plants in ranged from 0.069 to 0.27 g and from 0.17 to 0.53 g, respectively. The mean values for absolute stems and leaves dry masses in Chernozem and Arenosol in treatments of inoculated plants were 0.75±0.21 and 0.58±0.10 g/plant; while in treatments of non-inoculated plants were 0.18±0.06 and 0.36±0.09 g/ plant, respectively. These preliminary results indicate that the mycorrhizal fungi have a positive effect on plant growth and development. Further research should focus on studying how mycorrhizal fungi affects the content of essential oil, as well as the supply of phosphorus to plants in the soils examined by this study.

Keywords: Arenosol, Chernozem, winter savory, mycorrhizal fungi

## INTRODUCTION

Winter savory (*Satureja montana* L.) is perennial medicinal and aromatic plant belonging to Lamiaceae family. It grows in arid, sunny and rocky habitats of the sub-Mediterranean area. Winter savory is semi-evergreen subshrub growing to about 50 cm tall with the lanceolate leaves and white flowers that attract bees (Rzepa et al., 2012; Stepanović and Radanović, 2011). Genus *Satureja* counts about 30 species out of which 9 species have been registered in the area of central and western Balkans (Čopra-Janićijević et al., 2020). It is valuable plant, recognized for its therapeutic values. The leaves, flowers, and stems are used for herbal tea and, in traditional medicine, to treat various ailments (with carminative, digestive, expectorant, antidiuretic, etc. activities). Also, it is known as culinary herb due to flavor of its leaves (Ćetković et al., 2007; Rzepa et al., 2012).
Winter savory is highly variable plants specie with evident polymorphism within a single population and in populations coming from distant habitats (Slavkovska et al., 2001; Milos, et al. 2001). Variability is caused by cross-pollination leading to a large number of subspecies, varieties, and forms with different botanical characteristics and a broad chemical heterogeneity (Čopra-Janićijević et al., 2020). In family *Lamiaceae*, cross-pollination results heterozygous seeds from which heterogeneous plants arise with phenotypical variability and substantial phytochemical inconsistency (Kwon et al. 2006). Strategies within breeding programs to obtain phenotypical and phytochemical uniform individuals involve selection work, and also implementation of vegetative propagation techniques (Shetty, 1997), mostly by stem cuttings (Venkateshappa and Sreenath, 2013).
Biofetrilizers containing arbuscular mycorrhizal fungi (AM) are applied in agriculture in order to obtain benefits in yield, relying on mutual symbiotic association between a fungus and the root of cultivated plant. AM fungi of the phylum Glomeromycota are commercially most commonly used in agriculture, especially the species *Rhizophagus* (*Glomus*) *intraradices* and *Funneliformis* (*Glomus*) *mosseae* (Krüger et al., 2012). By applying these fungi, the plant more efficiently absorbs soil nutrients like phosphorus, zinc and copper (Burni and Hussain, 2011), increases resistance to drought and pathogenic diseases (Gupta et al., 2000; Koltai and Kapulnik, 2010). Also, these fungi improve the quality of the soil; when passing through the soil aggregates it positively affects the water-air regime of the soil (Hajnal-Jafari et al., 2012). Although AM is a naturally occurring phenomenon, colonization of plant roots is conditioned by a number of factors: the plant species and the type of fungus and environmental factors, among which low nutrient levels in the soil and poor plant nutrient supply are important (Chen et al., 2018).
In this research, the effects of mycorrhizal fungi (combination of *Glomus mosseae* and *G. intraradices*) were studied on growth and development of winter savory grown as a pot culture in a low carbonate Chernozem ("leached chernozems") with high clay content and typical Arenosol with low plant-available phosphorus content in both soil types.

## MATERIALS AND METHODS

### Production of rooted cuttings in laboratory conditions

Plant material used for the preparation of stem cuttings was one year-old shoots taken at the end of June 2020 from the 3-year-old mother stock plants, grown outdoors in a private collection in Stara Pazova, Serbia (44° 59′ 3.6″ N, 20° 9′ 23.4″ E). Softwood terminal stem cuttings, 13cm in length, were made. The leaves on the lower portion of the cuttings were removed, leaving 4 nodes. All cuttings were sterilized in 0.5% sodium hypochlorite for 10 min and then twice quickly rinsed with distilled water as recommended by Arango et al. (2012). The basal portion of cuttings (3cm) was treated with IBA (indole-3-butyric acid) based rooting powder ("Rhizopon AA powder 1%", Rhizopon®, Netherlands (IBA 1%). Following the application of rooting powder, the cuttings were put into the trays for propagation, filled with a cutting-specific medium (Steckmedium, Klasmann-Deilmann GmbH) of following characteristics provided by the manufacturer: a mix of peat moss and perlite, structure 0 - 6 mm, fertilizer NPK 12:14:24 -0.5 kg/m3, pH value 5.5 - 6.5, the EC value 15 mS/m (+/- 25 %). The trays with cuttings were kept inside a polythene tent (Grow Box) in the laboratory of the Institute for Medicinal Plants Research "Dr Josif Pančič", located in Belgrade, Serbia during the period June 25$^{th}$ to July 25$^{th}$, 2020, under the following growing conditions: the artificial lighting produced by cool fluorescent tubes (fluorescent Biolux 36W and Flora 36W), with a 16-hour photoperiod, providing up to 4,000 lux ≈ 54 μmol $m^{-2}$ $s^{-1}$ of photosynthetic photon flow density (PPFD); the relative humidity of 70 to 90% provided by intermittent automatic mist-propagation system, which was set to operate in following time-dependent modes: from 1st to 10th day - 5s/15 min, from 11th to 20th day – 5s/30 min, and from 21st to 30th day – 5s/90min; the air temperature was from 20°C to 23°C, while the substrate temperature was kept almost constant (23±2°C) (Svenson and Davies, 1995; Wilson et al.,2017; Mrđan et al., 2020). Monitoring of the relative humidity and the air temperature and in the Grow Box was provided by the use of HAXO-8 Data logger, while monitoring of the substrate temperature was provided by the use of Testo 110 NTC Thermometer.

### Hardening-off process, inoculation and experimental design

After 30 days period of rooting was concluded, the trays were taken outside the Grow box and left inside the laboratory, and were occasionally taken outdoor in order to ensure adaptation of cuttings to natural light irradiance, lower air temperatures and reduced relative air humidity of the outdoor environment. This acclimatization process (hardening - off process) of rooted cuttings started in Grow box by gradually decreasing the misting period during rooting of cuttings and lasted 10 days after trays have been taken outside the Grow box as recommended by Wilson et al. (2017). During the adaptation period the plants were watered with tap water. Rooted cuttings were transplanted into 1.5 L plastic pots (one plant per pot) filled with low carbonate Chernozem with high clay content and typical Arenosol taken from the plow layer of the soils in a disturbed condition. Soils used for filling pots were classified as Chernozem and Arenosol soil type according to The Classification of Yugoslav Soils (Škorić et al., 1985). Its main chemical and physical properties of soils are given in Table 1. Chernozem soil type originated from arable land in

Pančevo (N 44° 52' 40"; E 20° 42' 05"), while Arenosol originated from the The Deliblato Sands (N 44° 53' 05"; E 21° 04' 47").

Table 1. Main chemical and some physical properties of the plow layer (0 - 30 cm) for soil type 1(Chernozem) and type 2 (Arenosol)

| Soil type | pH | | $CaCO_3$ % | Humus % | N % | $P_2O_5$ mg/100g | $K_2O$ mg/100 g | Coarse sand % | Fine sand % | Silt % | Clay % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | KCl | $H_2O$ | | | | | | 2-0.2 mm | 0.2-0.02 mm | 0.02-0.002 mm | < 0.002 mm |
| 1 | 5.61 | 6.73 | 0.08 | 2.03 | 0.15 | 2.26 | 34.64 | 0.33 | 38.11 | 24.36 | 37.2 |
| 2 | 8.02 | 8.56 | 15.63 | 0.28 | 0.03 | 1.22 | 5.22 | 29.86 | 66.86 | 1.00 | 2.28 |

Experiment was established in Pančevo (44°52'20.0" N, 20°42'04.7" E), South Banat, Serbia. The potted plants were taken outdoors and placed on 1 mm thick water permeable black "agrotextil" film and arranged according to applied treatment. Treatments were potted plants with or without added inoculum (inoculated plants or non-inoculated plants/control) in different soil types (Chernozem and Arenosol): MCh-mycorrhiza in Chernozem; CCh- control in Chernozem; MAr-mycorrhiza in Arenosol; and CAr- control in Arenosol. In treatment with inoculated plants, the rooted cuttings were planted into a hole where the inoculum was previously added (15g of inoculum per plant) according to Arango et al. (2012). The used inoculum (Aegis Clay, Italpollina) is a mix of spores (25 spores/g of *G. mosseae* and 25 spores/g *G. intraradices*) and 60% of organic matter in powder formulation; structure < 1 mm, pH value 7. The experiment was set up in a split-plot design with 4 replications. The main plots were soil types, while sub-plots were 2 treatments (inoculated and non-inoculated plants) with 6 pots in a random arrangement in each repetition. Plants were watered regularly with an installed drip irrigation system with a flow rate 1L/h for each pot and weeds were regularly removed.

**Growth parameter**

Plants from each treatment were harvested at 60 days after inoculation with *G. mosseae* and *G. intraradices*. The absolute stems and leaves dry masses of plants from each treatment were recorded after have been dried at 105 °C during 48 hours to a constant mass.

## RESULTS AND DISCUSSION

Inoculated plants, in both soil types, had higher absolute stems and leave dry masses compared to the absolute stems and leaves dry masses in non-inoculated plants. The absolute stems and leave dry masses per plant in MCh and MAr ranged from 0.37 to 1.16 g and from 0.35 to 0.73 g, respectively; while in CCh and CAr ranged from 0.069 to 0.27 g and from 0.17 to 0.53 g, respectively. The mean values for absolute stems and leaves dry masses per plant in MCh and MAr were 0.75±0.21 and 0.58±0.10 g/plant, while of CCh and CAr were 0.18±0.06 and 0.36±0.09 g/plant, respectively (Figure 1).

Soil analysis show that in both soil types, Chernozem and Arenosol, low plant-available phosphorus and humus content were determined, while Arenosol lacks also in plant-available potassium (Table 1). Chernozem soil used in study is low in $CaCO_3$ and has high clay content. According to classification of soils shown by Pavlović, at al. (2017) in accordance with WRB classification (World Reference Base for Soil Resources), it is a variety of Chernozem (leached gleyed) characterized by less favourable mechanical composition, the absence of $CaCO_3$ in the humus-accumulative horizon and with the adsorption complex that exhibits slight acidification, accompanied by a decrease in pH. Although it is important to emphasize that soil with these properties, in large majority, refer to Chernozems in WRB; however, may also belong to Phaeozems due to lack of $CaCO_3$ in humus-accumulative horizon (Kabała et al, 2019). Due low water-retention capacity and low adsorption capacity, Arenosol soils have low productive value (Pavlović, at al., 2017).

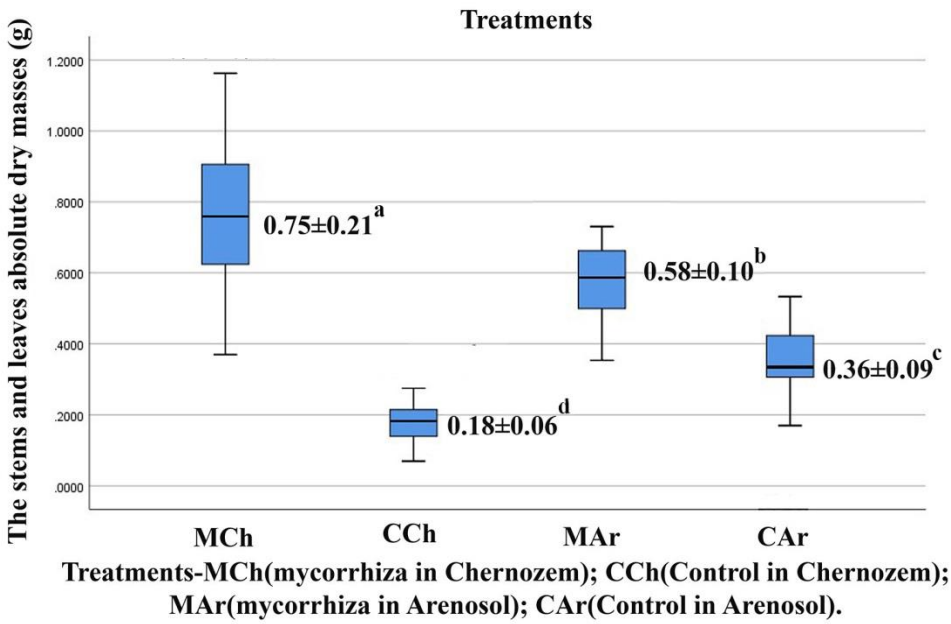


Figure 1. Comparative presentation of average ± standard deviation for absolute stems and leaves dry masses of *S. montana* [g/plant] with regard to fertilization models; Error bars denote standard deviation; means followed by the same letter are not significantly different at $p<0.05$

Hence, in this in this particular study, Chernozem has a better nutrient adsorption capacity, although less favorable mechanical composition, due to the high clay content, creates potential problems in land cultivation and in faster percolation of water into deeper layers. On the other hand, Arenosol with a sandy texture, has good physical properties in terms of air capacity and water percolation to depth, but has a very small capacity of adsorption of nutrients and water, and thus has limited fertility potential compared to Chernozem.

In order to achieve high yields in the production of cultivated crops on degraded soils, AM fungi are used as a tool wherein production of medicinal plants there are studies that

confirm the benefits of their use. AM fungi promotes the growth of medicinal plants and improves the production and accumulation of active ingredients of medicinal plants such as terpenes, phenols, and alkaloids (Zeng et al., 2013; Pandey et al., 2018; Piszczek et al., 2019). It also improves nutrition of plants, particularly in phosphorus (Lermen et al., 2019; Lazarević, et al., 2020) and helps plant to survive abiotic stressful conditions like water stress (Pirzad and Mohammadzadeh, 2018; Javan Gholiloo et al., 2019). In study of Khalediyan et al. (2021) on *Satureja hortensis* in a 2-year field trial conducted in Iran, showed that *G. mosseae* and *G. intraradices* fungi had an impact on dry mass of shoots, increasing it for 35,73 and 47.5 % in the first year and for 23.55 % and 62.84% in second year, respectively, in comparison to control. Carreón-Abud et al. (2015) investigated the effect of *Rhizophagus irregularis* inoculation on the growth of *Satureja macrostema* plants grown in soil: sand mix sterilized substrate (1.5 kg, v/v, 3:1, pH 5.5) in greenhouse, where after 60 days shoot dry mass was 18.4 g/plant in mycorrhizal plants and 8.8 g/plant in non-mycorrhizal plants. Arango et al. (2012) in study done on *Mentha piperita* L. showed that 40 days after inoculation, used *Glomus intraradices A4 and G. intraradices B1* had increased shoot dry mass and was 0.61g and 0.54g, respectively, compared to control (0.41g), which was similar to our results for *S. montana* grown in Arenosol (Figure 1). It is noted that plants in Chernozem control treatment had the lowest absolute stems and leaves dry mass. It is assumed that the plant species, taking into account its natural habitat, prefers porous soil types. Thus the growth of pot winter savory plants in Arenosol control is proven to be better concerning non-inoculated plants in Chernozem (Figure 1). It can be assumed that increment of the aboveground plant parts in inoculated plants in Chernozem is result of AM fungi being able to enhance plant nutrition by external hyphal network of AM fungi providing better contact with soil particles and increasing effective root surface, in combination with the existing adsorption capacity of Chernozem soil (Bagyaraj, et al., 2015) although it has less favourable mechanical composition due to high clay content (Table 1).

## CONCLUSION

This study was conducted in order to determine how suitable is *S. montana* for cultivation on degraded soils and to which extent will mycorrhizal fungi contribute to better and more efficient land use and expansion of the cultivation area of the studied species to areas with reduced soil fertility. The preliminary results indicate that the mycorrhizal fungi have a positive effect on plant growth and development. Further research should focus on studying how mycorrhizal fungi affects the content of essential oil, as well as the supply of phosphorus to plants in the soils examined by this study.

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