Journal of Applied Horticulture, 24(2): 130-134, 2022

DOI: https://doi.org/10.37855/jah.2022.v24i02.25

Journal of Applied Horticulture

ISSN: 0972-1045

The impact of Myconate® HB (formononetin) on the growth, chemical composition and content of active compounds in herbal sage *Salvia officinalis* L. and *Valeriana officinalis* L.

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Abstract

The research aimed to demonstrate the effect of Myconate® HB (formononetin) on the growth, weight, nutrients and active compounds in *Valeriana officinalis* and *Salvia officinalis*. The research was carried out in the experimental field on brown soil. A univariate experiment was established using the randomized block design in four replications. The research showed that the use of Myconate® (Agrii Polska Sp. z o.o.) on *V. officinalis* seedlings positively affected the weight of the aerial parts and roots, as well as the content of dry matter and fiber. However, the content of total protein and nitrogen-free extracts was unfavourable. In the case of *S. officinalis,* the use of Myconate® also had a positive impact on the weight of the aboveground parts and roots and on the content of total protein and crude ash in the herb, while it negatively influenced the contents of dry matter, fiber and nitrogen-free extracts. Myconate®, however, did not affect the length of the phenological stages in the experimental plants. The percentage content of essential oils increased during the vegetation period in both species in all the tested plots, while their statistically higher contents were observed from the full tillering stage in the plots sprayed with Myconate®.

Key words: Seedling stimulation, plant development, essential oils, *Salvia officinalis*, *Valeriana officinalis,* Myconate® HB, formononetin

Introduction

One approach to reduce the reliance on agricultural chemicals is by introducing beneficial biological organisms into the soil. This treatment aims to safeguard plants from pathogens and create favorable conditions for their growth and development, as supported by studies by Aparecida *et al.* (2015), Barea (2015), Davies *et al.* (2005), and Pal *et al.* (2016). Microbiological preparations, containing carefully chosen, advantageous microorganisms commonly found in nature, have proven highly effective (Castillo *et al.,* 2016). Among the popular microbiological preparations used in plant cultivation are atmospheric nitrogen-fixing inoculants and mycorrhiza (Kosicka *et al.,* 2015; Nowak and Kunka, 2009 and Schreiner, 2005).

According to Pytlarz-Kozicka *et al.* (2017), the method of mycorrhizal application and their technical and technological characteristics are important for their effectiveness. Selecting a proper technology of liquid and soil-injecting fertilisation with preparations chosen depends on the plant growth phase. In case of combining soil and foliar fertilization with mycorrhizae, the temperature and humidity of the substrate are important. These factors significantly impact the rate of nutrient release from fertilisers. The use of mycorrhizal innocuations has a positive effect on the environment, as it improves the flow of water and nutrients in the soil environment, which leads to faster plant growth (Jamiołkowska *et al.*, 2018; Kosicka *et al.*, 2015; Nowak and Kunka, 2009).

The development of mycorrhizal fungi is hampered by high mineral fertilization, especially with nitrogen and phosphorus or fresh organic fertilizers in high doses (Kowalska *et al.*, 2015). Chemical plant protection products, especially fungicides, are also harmful to mycorrhizae. Adverse climatic and soil conditions, such as droughts or excessive rainfall, also have negative impact. Furthermore, chemical seed treatment is not conducive to the development of mycorrhizae in the first period of plant growth (Wright and Anderson, 2000).

Most of the studies related to the use of mycorrhizal fungi concern ornamental and fruit plants (Kowalska *et al.*, 2015; Nowak and Kunka, 2009; Wright and Anderson, 2000), while few reports describe the effect of mycorrhizae on other useful plants, *e.g.,* vegetables or herbs (Tartanus, 2017). In aromatic plant production, arbuscular mycorrhizae (AM) are indicated as the ones that significantly increase the yield and the content of aromatic oils in plants (Tarraf *et al.*, 2015).

Essential (aromatic) oils are natural compounds found in various parts of plants. They are mixtures of various chemical compounds, including alcohols, ketones, esters, terpenes and aldehydes, are primarily found in stems, leaves and flowers in glands or oil hairs (Loomis and Corteau, 1972). Essential oils are an important element in fighting pests but also influence the growth and development of other accompanying plants (negative and positive allelopathy).

The research aimed to evaluate the effect of Myconate® HB on seedlings with a mycorrhizal preparation stimulating the formation of mycorrhizae and consequently the plant development, nutrient content and weight percentage of essential oils in *Valeriana* *officinalis* and *Salvia officinalis* in field cultivation under the medium soil conditions.

Materials and methods

Growing conditions: The field experiment with *S. officinalis* and *V. officinalis* was conducted in 2016–2017 in Pawłowice (51°09´ N, 17°06´ E), in a field belonging to the Wrocław University of Environmental and Life Sciences in Wrocław. In spring, before sowing, mineral fertilization was applied in the dose: 40 N–40 P–60 K as pure components. The experiment began in the third week of April. Experimental plants were cultivated in an area of 8.0 m^2.

The field experiment was established on a soil classified as a good rye complex, valuation class IVb. The soil was slightly acidic, the phosphorus content was very high, and the potassium and magnesium content was medium. The soil conditions were optimal for both experimental species (Berbeć 1968; Moniuszko and Wiśniewski, 2011) and mycorrhizal fungi (Hilszczańska, 1997).

Study site and experimental design: Univariate experiments were established using the randomized block design in four repetitions. The variants for both plants were as follows: a) control – without stimulation and b) sprayed with Myconate® HB (95 %). The Myconate® HB substance used in the experiment is not a typical mycorrhizal preparation but one of the few preparations stimulating the formation of mycorrhizae. The active substance here is the isoflavonoid formononetin (4'-methoxy, 7-hydroxy isoflavonoid) – 95 %, originally obtained from the roots of clover subjected to the stress of phosphorus deficiency (Aparecida *et al.*, 2015). Formononetin, as an active compound, is applicable to the industries of food, medicine, cosmetics and agriculture (Machado Dutra *et al.*, 2021)

The benefits of using Myconate® HB, as stated by the manufacturer (Agrii Polska Sp. z o.o.), are: increased root mass, higher resistance to drought, effective uptake of nutrients – mainly phosphorus (also phosphorus naturally present in soil) and micro-nutrients, better atmospheric nitrogen fixation by legumes, reduced invasiveness of pathogens and soil protection through increasing the durability of soil aggregates and improved soil structure due to glomalins produced by fungi (Agrii www.agrii.pl › myconate-hb-new).

Analysis: Three weeks before sowing, the germination energy and capacity of seeds of both species were tested (for *V. officinalis* according to the Polish Standard PN-65950, while for *S. officinalis* according to ISTA (1995)). Throughout the growing season, observations of the growth and development of both experimental plants were conducted and infections with pathogens were recorded. Weed control was performed manually. Before harvest, samples of *S. officinalis* and *V. officinalis* were collected for biometric tests (5 plants per plot), determining the weight of the aerial parts and roots. For chemical analyses, samples of 3 × 100 g were taken from each plot. The dry matter content was determined by the drying method (at 105°C for 4 hours). The total protein, crude ash, crude fibre and nitrogen-free extracts (NFE) content was performed using the reflectance spectroscopy in the NIRS (InfraAlyzer 450s, Bran+Luebbe, Norderstedt, Germany). The weight and volume percentage of essential oils in the herb *V. officinalis* and *S. officinalis* was determined according to the scale of the Deryng apparatus (WPL Gliwice, Poland). The volume of the obtained oil was converted equivalent to 100 g of raw herbal material, expressed as a percentage by weight and volume. The results were statistically analyzed using variance analysis with the AWA software for univariate randomized block experiments. The dependence of the studied variables was determined based on the Fisher test with the error probability $\alpha = 0.05$.

Results and discussion

Weather conditions: The growing seasons in which the research was conducted were very diverse regarding air temperature and precipitation (Table 1). They were characterised by a slightly higher temperature and uneven precipitation compared to the long-term average. The insufficient rainfall during the plant emergence period made them weak and inconsistent. The lack of rain in June and August and its excess in July were not conducive to the development of plants. Hot and dry August contributed to the inhibition of growth but positively influenced the production of essential oils in experimental plants.

In his experiments with Valerian root and its water requirements, Berbeć (1968) stated that this species is resistant to drought and moderate humidity provides higher yields. He also found that the content of essential oils decreased along with increasing humidity.

Sage is a thermophilic plant, hence the level of its yield and the quality of the raw material are determined, apart from agro-technical factors, by the weather conditions during the vegetation season (Bielski *et al.*, 2011). In a study carried out in Finland, Galambosini *et al.* (2002) found that in the south of the country, sage yields are 10-40 % higher than in plantations 500 km to the north. Moreover, a shorter growing season has a greater effect on biomass production than on the content and composition of the oil. Prodan and Tabără (2010) reported that the length of the growing season has a greater impact on biomass production than on the content and composition of essential oil in sage. In an experiment conducted with *S. officinalis*, Berbeć (1968) observed that this species is drought resistant and higher herb yields can be obtained in moderate humidity conditions, while the content of essential oils decreases along with increasing humidity.

Mycorrhizae: The progressive degradation of the environment, which is the result of, among others, the use of mineral fertilizers and

Table 1. Weather conditions during the growing season

Year	IV	V	VI	VII	VIII	IX	X	Mean IV–X
			Mean air temperature (°C)					
2016	8.7	15.3	18.6	19.5	17.9	16.4	8.5	14.98
2017	7.9	14.2	18.5	19.0	19.4	13.3	12.0	14.90
1986–2015	8.9	14.4	17.3	19.6	18.6	13.7	9.1	14.51
			Total precipitation (mm)					Total
2016	46.4	5.3	44.6	114.3	27.1	44.7	83.8	366.2
2017	57.0	24.1	52.5	112.2	43.6	65.7	71.4	426.5
1986–2015	33.6	54.1	67.4	78.9	65.3	44.9	33.7	377.9

chemical protection agents in plant cultivation, resulted in the need to introduce alternative protection methods (Borkowska, 2002). Mycorrhizae are a common phenomenon (it affects about 85 % of vascular plant species) and, at the same time, it is necessary for the proper functioning of plants. Mycorrhizae improve the general condition of the plant, support its growth, increase resistance to pathogens and contribute to more effective uptake of nutrients from the soil (Dood, 2000; Santos *et al.*, 2020; Tartanus, 2017; Jamiołkowska *et al.*, 2018). Recently, the important role of mycorrhizal fungi in the detoxification processes of heavy metals in plants from areas with naturally high heavy metal contents and/or contamination has also been pointed out (Krupa, 2010). Endomycorrhizae dominate among herbaceous plants. The degree of root colonization by endomycorrhizal fungi and their activity varies during vegetation and depends on weather conditions. Schroeder and Janos (2005) observed that plant mycorrhization declines with heavy rainfall.

Plant development. Our research showed no effect of the application of Myconate® HB on the individual stages of vegetative growth of valerian and medicinal sage. The length of phenological stages (Table 2) depended on the weather conditions in the year of the experiment and was slightly longer in 2017. Xavier and Boyetchko (2002), who studied the morphology of plants exposed to mycorrhizal fungi, did not find their impact on the length of the phenological phases. However, they reported increased root colonization until the tillering phase and signals increased spore production by non-root hyphae.

Table 2. Length of phenological stages (number of days) of *Salvia officinalis*

Phenological stage	Control		Myconate HB	
Salvia officinalis				
Sowing	19.04.2016	22.04.2017	19.04.2016	22.04.2017
Seedling emergence	18	20	18	18
Full emergence	4	6	4	4
Leaf emergence	6	7	5	6
Tillering	29	31	29	30
Shoot formation	24	24	24	22
Plant withering	–	–	–	–
Harvest	11.10.2016	18.10.2017	11.10.2016	18.10.2017
Valeriana officinalis				
Sowing	18.04.2016	21.04.2017	18.04.2016	21.04.2017
Seedling emergence	22	24	22	23
Full emergence	6	6	6	5
Leaf emergence	16	17	15	17
Tillering	12	13	14	14
Shoot formation	8	9	9	10
Plant withering	64	69	66	69
Harvest	10.10.2016	17.10.2017	10.10.2016	17.10.2017

Energy and germination capacity: The high quality of the seed material is one of the most important factors affecting the quality and size of the crop. While examining the germination energy and capacity of *S. officinalis* and *V. officinalis* (Table 3), it was found that slightly better parameters were achieved in both species in 2017. Inoculation with mycorrhizal fungi after sowing or during the seedling production period was favourable for developing the plants' root systems. Tarraf *et al.* (2015) and Tartanus (2017) observed that plants with mycorrhizal root systems showed a marked increase in the weight of the aerial parts compared to those without rhizosphere inoculation with mycorrhizal fungi.

Table 3. Energy and germination capacity

Species	Germination energy [%]		Germination capacity [%]	
	2016	2017	2016	2017
S. officinalis	49	56	77	78
V. officinalis	48	54	88	93

Plant productivity: Gosling *et al.* (2006), Koide and Mosse (2004), Lekberg and Koide (2005) and Ribeiro *et al.* (2016) found that in plant cultivation, mycorrhizae are beneficial by accelerating plant growth and increasing the yield of both the above-ground mass and the root system. The results of our research confirm these observations because both in sage and valerian, the weights of the aerial parts and roots were significantly higher when Myconate® HB was applied (Table 4).

Table 4. Plant mass before harvest (average for one plant)

Species	Stimulation	Aerial parts' mass [g]		Root mass [g]		Plant mass [g]	
		2016	2017	2016	2017	2016	2017
Salvia officinalis	Control	289.3	316.4	129.3	136.8	418.6	453.2
	Myconate HB	322.4	342.1	163.1	171.0	485.5	513.1
	NIR = α 0.05	13.1	16.4	15.4	13.2	12.5	10.1
	Mean for years	305.9	329.3	146.2	153.9	452.1	483.2
Valeriana officinalis	Control	192.8	203.4	133.0	137.1	325.8	340.5
	Myconate HB	242.1	249.7	167.0	180.8	409.1	430.5
	NIR = α 0.05	7.6	6.4	3.9	4.2	9.5	9.1
	Mean for years	217.4	226.6	150.0	159.0	367.4	385.5

The content of nutrients in the dry matter of *S. officinalis* and *V. officinalis* in our research depended primarily on the weather conditions during the vegetation period. It was found that stimulation with Myconate® HB contributed to a significant increase in the total protein content only in the case of *S. officinalis*, while the crude fibre content in dry matter of *Salvia officinalis* and NFE compounds were higher in the control samples in both species (Table 5).

The percentage of most components in the green matter of *V. officinalis* did not differ significantly between both species. Only an increase in the dry matter and crude fiber content was observed because of Myconate® HB application (Table 6). In the case of *S. officinalis*, on the other hand, the percentage of total protein was significantly higher where the Myconate® HB preparation was applied. The dry mass, crude ash and fibre content was significantly lower after formononetin application.

Candido *et al.* (2013) reported an increase in the dry matter content in tomato fruit as a result of plant mycorrhization. The authors also observed an increase in the fruit's nitrogen content due to mycorrhization. However, they did not note the effect of

Table 5. Nutrient content in dry matter (mean for years)

Species	Stimulation	Total protein	Crude ash	Crude fibre	Crude fat	NFE
Salvia officinalis	Control	12.6	12.3	20.7	6.08	48.4
	Myconate HB	13.8	12.5	20.3	6.00	47.3
	NIR = α 0.05	0.2	n.s.	0.2	n.s**.	0.4
Valeriana officinalis	Control	15.5	14.9	15.4	4.10	50.0
	Myconate HB	14.9	14.8	16.9	3.92	49.5
	NIR = α 0.05	0.2	n. s.	0.4	n. s.	0.1

NFE= Nitrogen free extracts. n.s.=not significant this treatment on the total sugar content in tomato fruit compared with the control. Kowalska *et al.* (2015) found a high yield of tomatoes, higher ascorbic acid contents and total soluble sugars in fruits harvested from inoculated plants grown on mineral wool.

When examining pepper fruits, Tartanus (2017) did not find any significant effect of mycorrhizae on content of the analyzed nutrients. He noticed only a slight increase in sugars in fruits harvested from mycorrhizal plants compared to those grown without mycorrhizae. Borowy and Matela (2012), as well as Golcz and Bosiacki (2008), observed a significant decrease in the fresh and air-dry mass of the thyme herb after using the mycorrhizal innocuation as compared without mycorrhizae.

Table 6. The percentage content of nutrients in green matter (Mean of two years)

Species	Stimulation	Dry mass	Total protein	Crude ash	Crude fiber	Crude fat	NFE
Salvia officinalis	Control	39.2	2.90	7.19	8.42	2.46	18.2
	Myconate HB	38.1	3.95	6.35	7.54	2.38	17.9
	NIR = α 0.05	0.3	0.3	0.2	0.2	n. s.	n. s.
Valeriana officinalis	Control	18.9	2.94	2.82	2.92	0.77	9.45
	Myconate HB	19.1	2.84	2.82	3.22	0.75	9.52
	NIR = α 0.05	0.1	n. s.	n. s.	0.1	n. s.	n. s.

NFE=Nitrogen free extracts. n.s.=not significant

Essential oils: Our research determined that the percentage of essential oils in *S. officinalis* and *V. officinalis* depended on the stage of development and the weather conditions during the vegetation period and increased significantly along with the progress of vegetation (Table 7). A significantly higher content of oils due to spraying with Myconate® HB was observed from the full tillering phase in both tested species. A similar tendency was observed by Tarraf *et al.* (2015), who, by chromatographically analyzing sage and thyme, showed a greater amount of oil in the leaves of plants inoculated with mycorrhizal fungi, as compared to the control. After applying mycorrhizal fungi, Golcz and Bosiacki (2008) observed a reduction in the essential oil content of thyme herb compared to the untreated raw material. Similarly, Smith and Read (2008) and Loomis and Corteau (1972) reported that arbuscular mycorrhizal (AM) fungi establish symbiotic associations with plants, facilitating the absorption of phosphorus, which significantly influences the biosynthesis of essential oils.

Table 7. Percentage by weight and volume (determined according to the Deryng apparatus scale) of essential oils in the herbs depending on the phenological stage

Species	Phenological stage	2016			2017		
		Control	Myconate HB	NIR = α 0.05	Control	Myconate HB	NIR = α 0.05
S. officinalis	Tillering beginning	0.58	0.59	n.s.	0.76	0.78	n.s.
	Full tillering	0.64	0.67	0.02	0.81	0.86	0.03
	Before harvest	0.75	0.83	0.04	0.84	0.89	0.02
	NIR = α 0.05	n.s.	0.1	–	0.1	0.1	–
V. officinalis	Tillering beginning	0.34	0.33	n.s.	0.41	0.38	n.s.
	Full tillering	0.56	0.63	0.06	0.53	0.67	0.1
	Before harvest	0.57	0.61	0.03	0.55	0.64	0.08
	NIR=α 0.05	0.1	0.1	–	n.s.	0.1	–

n.s.=not significant

The rhizosphere and mycorrhizae often determine the proper growth of plants. Microorganisms create a specific biological environment characteristic for a given plant around the roots, favouring its development.

Our research aimed to determine the effect of seedling stimulation with Myconate® HB confirmed its beneficial effect on *S. officinalis* and *V. officinalis*. Myconate® HB increased the plant mass, content of essential oils, and chemical composition of both species. Mycorrhizal inoculation is, therefore a promising technology in a sustainable agricultural system in improving plant productivity.

Acknowledgments

This research was carried out in the Institute of Agroecology and Plant Production, Wrocław University of Environmental and Life Sciences, with no specific grant from funding agencies of the public, commercial, or not-for-profit sectors.

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Received: October, 2021; Revised: January, 2022; Accepted: February, 2022