Hungarian University of Agriculture and Life Sciences

Factors influencing the intraspecfic and interspecific variabilities of lavender species (*Lavandula angustifolia* Mill. and *Lavandula* × *intermedia* Emeric ex Loisel)

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1. HISTORY OF THE WORK, OBJECTIVES

Lavender, although a medicinal plant from European Mediterranean regions, has been cultivated and used for drug production across the world (Bulgaria, France, Spain, Portugal, United Kingdom, China, Australia, United States of America, etc.). *Lavandula angustifolia* (true lavender) and *Lavandula × intermedia* (hybrid lavender) species are the best known in cultivation in Hungary. The latter is a spontaneous hybrid of *L. angustifolia* and *L. latifolia*.

In addition to the traditional use of lavender as a sedative, antispasmodic and digestive stimulant, the growing demands of the pharmaceutical, perfume, cosmetic and, more recently, the food industry justify the production of its drugs in extensive production areas (Bernáth, 2013). The list of the diverse therapeutic indications of the plant is also extensive, and an increasing number of research projects confirms the sedative effects of its essential oil helping the process of falling asleep (Sváb et Heltmanné, 2000;). Its great advantage is that its medicinal use is safe in therapeutic doses and has fewer side effects than other synthetic sleeping pills and sedatives (Böszörményi, 2018).

Demand for high-quality, safe and efficient essential oils has increased among both growers and customers and this results in the use of the appropriate lavender varieties and the introduction of modern and sustainable cultivation technologies. However, the development of lavender cultivation technology focuses not only on the extractable essential oil, but also on phenolic compounds, which have been proved to have antioxidant effects (Blažeković et al., 2010). Nowadays, artificial antioxidants are gradually replaced by antioxidants from natural sources extracted from medicinal plants. Based on the above, it is important to be aware of the factors that may influence the accumulation of these active ingredients, both volatile and non-volatile, within the lavender. Therefore, one of the primary objectives of our work is to identify the effects of endogenous and exogenous factors affecting the intraspecific and interspecific variability of true and hybrid lavender, in terms of its essential oil content and composition and also the values of all polyphenols and antioxidant capacities. Answers to the following questions were sought in the course of our research:

- Are there any significant intraspecific differences between the true and the hybrid lavender varieties regarding the amount and composition of the active ingredients?
- Is there any difference between the two economically most important lavender species regarding their reaction to the changes of internal and external factors?
- How can individual development (flowering phases) and organ diversity distribution affect the quality of drugs?

- To what extent may external factors, weather (year) and habitat conditions influence compositional parameters?

2 MATERIAL AND METHODS

2.1 Location and time of experiments

Experiments were carried out in the Lavender Farm at Dörgicse and in the Lavender Farm at Szomód as well as in the experimental variety collection planted in the Medicinal Plant Production Unit of the Experimental Station, Hungarian University of Agriculture and Life Sciences (MATE) in Budapest, between 2017 and 2019.

2.2 Plant material of the experiments

The experiment included the simultaneous evaluation of *L. angustifolia* **’Hidcote’** and **’Munstead’** as well as *L. x intermedia* **’Grappenhall’** and **’Grosso’** varieties at the production sites at Dörgicse and Szomód. These studies were completed with plant sampling from further species: in addition to the above **’Aromatico Silver’**, **’Beate’**, **’Budakalászi’** varieties were involved in the experiment at Dörgicse and **’Judit’** variety at Szomód. The lavender variety collection of the Experimental Plant at Soroksár included the following varieties: ’Budakalászi’, ’Hidcote’, ’Maillette’, ’Munstead’, and ’Tihany’ true lavender and ’Grappenhall’, ’Grosso’, and ’Judit’ hybrid varieties. Measurements were performed on productive stocks of different ages.

2.3 Methods of open-field observation and measurements

2.3.1 Evaluation of the differences between species and varieties

To compare the essential oil properties of each lavender variety, we sampled the stocks at the Dörgicse production site during the summer harvest period in 2017 and 2018. Six *L. angustifolia* (’Aromatico Silver’, ’Beate’, ’Budakalászi’, ’Hidcote’, ’Maillette’, and ’Munstead’) and two (’Grappenhall’ and ’Grosso’) varieties were involved in the experiment.

We also looked at the manifestation of intraspecific diversity for total polyphenol content (TPC) and antioxidant capacity (FRAP). The lavender varieties were analysed in the production sites where we were able to collect the most of them, therefore *L. angustifolia* varieties ('Aromatico Silver', 'Beate', 'Budakalászi', 'Hidcote', 'Maillette', and 'Munstead') were examined at Dörgicse, while *L. × intermedia* varieties ('Grosso', 'Grappenhall' and 'Judit') at Szomód for two experimental years, in the summer of 2017 and 2018, at full flowering.

2.3.2 Methods of ontogenetic examinations

Changes in the essential oil properties (essential oil content and composition) of lavender varieties in various flowering stages were assessed in Soroksár in 2018 and 2019. The varieties included in the study were four *L. angustifolia:* 'Budakalászi', 'Hidcote', 'Maillette', and 'Munstead', and three *L.* × *intermedia* varieties: 'Grosso', 'Grappenhall' and 'Judit'. The samples were taken in two flowering stages (full flowering and after flowering) in both years from all varieties, while the green bud phase was evaluated in 2018-2019 for 1 variety of each species ('Budakalászi' and 'Grosso'), and the purple bud phase was recorded in the productive stocks in their 3rd year in 2019. We also examined the effect of flowering stages on the total polyphenol content (TPC) and antioxidant capacity (FRAP) values of lavender varieties in the collection of Soroksár in two experiment years (2018 and 2019). Samples for these experiments were taken in full flowering and in after flowering phenophases, consisting of three *L. angustifolia* ('Budakalászi', 'Hidcote', and 'Munstead') and three *L.* × *intermedia* ('Grosso', 'Grappenhall', and 'Judit') varieties. Since the plantation still young in 2018, we only had the opportunity to take samples from the purple bud phase in 2019.

2.3.3 Methods of examining the effects of organ diversity

In order to analyse the effects of organ diversity, flower and leaf samples of two *L. angustifolia* ('Hidcote' and 'Munstead') and two *L.* × *intermedia* ('Grappenhall' and 'Grosso') varieties were taken in the autumn of 2016 (24th October), in the summer of 2017 *(L. angustifolia*: 18th June and *L.* × *intermedia*: 14th July) and in the autumn of 2017 (31st October).

2.3.4 Methods of assessing the effects of year

The analysis of the effects of the years 2018 and 2019 on both the essential oil content and composition, as well as the development of phenolic compounds and antioxidant capacity was carried out in the stock at Soroksár, sampling was always carried out in full flowering. In addition, the analysis of essential oil properties took place in the summer of 2017 and 2018 at the Dörgicse production site, where three *L. angustifolia* varieties ('Budakalászi', 'Hidcote', and 'Munstead') were included in the comparison, also in the stage of full flowering.

2.3.5 Methods of examining harvest time

In 2017, we were able to harvest in two different periods in one growing season in the case of two *L. angustifolia* ('Hidcote' and 'Munstead') and two *L.* × *intermedia* ('Grappenhall' and 'Judit') varieties. In doing so, we revealed differences between the intrinsic materials of the samples taken during the summer (*L. angustifolia*: 18th June and *L.* × *intermedia*: 14th July) and autumn (31st October) flowering periods.

2.3.6 Methods of assessing the effects of production site

For studying the effects of the production site, two varieties *of L. angustifolia* ('Hidcote', 'Munstead') and two *L.* × *intermedia* ('Grosso', Grappenhall') were evaluated in terms of essential oil content and composition and also in terms of the phenolic compounds. In our experiment, we compared data of samples taken from two different production sites (Dörgicse and Szomód) in the summer of 2017 during the stage of full flowering (*L. angustifolia* samples taken on 18th June, *L.* × *intermedia* samples taken on 14th July).

2.4 Essential oil content measurement

The dried plant material (5-10 g) was distilled by a Clevenger laboratory device in accordance with the specifications of the 7th edition of The Hungarian Pharmacopoeia (1986). The duration of water vapour distillation was 1 hour. I applied 3 repeats for each item. The amount of essential oil was expressed in ml/100 g in relation to the anhydrous dry matter content of the drug.

2.5 Gas chromatographic analysis of essential oils

For analysing the components of essential oils GC-MS method was applied. Component identification was performed on the basis of their mass spectrum with the help of NIST library and our own essential oil library using retention times and indices (Adams, 2007). Three parallel measurements were performed for each sample.

2.6 Total polyphenol content measurement

Total polyphenol content (TPC) measurement was carried out based on the method of Singleton et Rossi (1965). Absorbance was measured with a Thermo Evolution 201 spectrophotometer at λ=760 nm. Three parallel measurements were performed for each sample. Total phenol content was determined from the measured absorbance using a curve calibrated for gallic acid and the results are given in mg GAE/g DW.

2.7 Antioxidant capacity measurement

For the determination of the total antioxidant capacity of the lavender samples, the modified method of Benzie et Strain (1966) was applied with three parallel measurements in this case as well. Absorbance was measured with a Thermo Evolution 201 spectrophotometer at λ=760 nm. A calibration curve was created for the measurement in advance using ascorbic acid as a known compound. The value of the concentrate is given in mg AAE/g DW.

2.8 Methods of the biometric assessment of the experiments

Results were analysed with single factor variance analysis using the software IBM SPSS Statistics 25. The normality of the data was verified by the Kolmogorov-Smirnov test and the standard deviation homogeneity was verified by Levene test. In the case of standard deviation homogeneity, Tukey HSD post hoc comparison was carried out for the comparison of value pairs. In case the standard deviation homogeneity did not match, the data pairs were compared using the Games-Howel test. The data were evaluated at a confidence level of 95 % ($p \leq 0.05$).

3 RESULTS

3.1 Effects of endogenous factors on the interspecific and intraspecific variability of lavender

3.1.1 Effects of genotype on the chemical variability of lavender

Based on our results, the interspecific variability of *L. angustifolia* and *L.* × *intermedia* was demonstrated in a significant number of the studied parameters. In terms of essential oil content, a significant difference was detected both between the two species ($p<0.0001$) and between the varieties ($p<0.0001$). It is important to note that not all varieties show a high degree of variability depending on the production site and year: the essential oil content has not changed significantly in different samples in the case of 'Aromatico Silver', Maillette', and 'Munstead', thus the stability of these varieties is more favourable.

The highest essential oil content (8.2 ml/100g) was found in a true lavender variety 'Budakalászi', which also exceeded the essential oil content of hybrid lavender varieties ('Grosso': 7.7 ml/100g) in our experiment. The smallest essential oil content was found in the true lavender variety 'Beate' (1.9 ml/100g).

Regarding the composition of the essential oil, both species are characterised by a significant manifestation of the variety effect, which is more significant in the hybrid lavender of the two species.

Higher TPC and FRAP values were obtained for hybrid lavender varieties than for true lavender varieties. The strongest antioxidant activity was detected in the inflorescences of 'Grappenhall' (204.7 mg AAE/g DW). Lowest antioxidant capacity values were detected in ‘Aromatico Silver’, a true lavender variety (81.3 mg AAE/g DW).

Regarding the studied intrinsic parameters, only the antioxidant capacity values of the *L.* × *intermedia* species were not affected significantly by the species in 2017.

3.1.2 Effects of flowering stages on the chemosyndromes of lavender

During flowering, significant differences can be detected regarding essential oil content and composition. We found that in terms of the highest essential oil accumulation and the most favourable composition, the harvest period of true and hybrid lavender should be in different flowering stages. *L. angustifolia* and *L.* × *intermedia* showed the highest essential oil content and linalool proportion after flowering and in full flowering respectively. The effect of flowering stages on volatile components was therefore characteristic for the species.

Phenophases played little role in the accumulation of polyphenols. There were several stable varieties ('Munstead', 'Grappenhall') from both species in our experiment, the total polyphenol content and antioxidant capacity values of which did not change significantly in different flowering stages. The interaction between variety and flowering stage factors for polyphenol content was statistically verifiable (flowering stage*variety interaction: $p<0.001$).

3.1.3 Total polyphenol content and total antioxidant capacity compared to organ diversity

The inflorescence and leaf parts of the lavender species show significant differences mainly in the case of the TPC and FRAP values of *L.* × *intermedia* varieties.

The difference between the TPC and FRAP values of plant organs was also strongly related to the seasons in our experiment, as significantly higher values were found in the flower samples during the summer cutting ('Grappenhall': TPC: flower: 140.8 mg GAE/g DW; leaf: 126.8 mg GAE/g DW; FRAP: flower: 150.3 mg AAE/g DW; leaf: 127.5 mg AAE/g DW), and in the leaf samples at the time of the autumn cutting ('Grappenhall': TPC: flower 244.3 mg GAE/g DW; leaf: 311.7 mg GAE/g DW; FRAP: flower: 140.6 mg AAE/g DW; leaf: 196.7 mg AAE/g DW).

3.2 Effects of exogenous factors on the interspecific and intraspecific variability of lavender

3.2.1 Effects of production year on the chemical variability of lavender

The effect of production year influenced significantly the essential oil content of both lavender species in the experiment at Soroksár (*L. angustifolia* varieties: 'Budakalászi': $p<0.018$; 'Munstead': $p<0.0001$; 'Maillette': $p<0.0001$; *L.* × *intermedia* varieties: 'Grappenhall': $p<0.034$; 'Judit': $p<0.004$).

Regarding the composition of the volatile components, significant differences can be detected in the samples of different years in the case of both species: the effects of the given year were detected for several components in the case of *L.* × *intermedia* species (e.g., 1.8-cineole, linalool, camphor, α-terpineol, linalyl acetate).

Exogenous effects, including effects of the production year on the accumulation of phenoloids were also detected in both species of lavender. In our present work, we have also proved the interaction of year and variety effects in the TPC and FRAP values (year*variety effect: $p<0.001$).

In terms of the total polyphenol content values, the greatest difference between the two experimental years was measured in the hybrid lavender variety 'Judit' (134.1 mg GAE/g DW) while the smallest difference between the two years was shown by the true lavender variety 'Budakalászi' (26.9 mg GAE/g DW).

3.2.2 Effects of harvest time on the total polyphenol content and the total antioxidant capacity values of lavender varieties

When assessing the effect of different harvest times (summer and autumn) within one year, we found that for all varieties there is a significant difference between the compositional indicators of inflorescences (TPC, FRAP), with the exception of the hybrid lavender variety 'Grappenhall'. For each significant difference, higher values were found in the samples of the autumn cut. The effect of harvest time on TPC values, similarly to the effect of the production year, was different for each species and variety (harvest time*variety: TPC: $p<0.020$).

3.2.3 Effects of production site on the chemical variability of lavender

Based on our experiments, we found that there were also differences between lavender varieties in how they responded to the effects of the production site. The essential oil content of the true lavender variety 'Munstead' showed only minor fluctuations at different production sites (2017: **Dörgicse**: 3.6±0.3 ml/100g, **Szomód**: 3.6±0.5 ml/100g; 2018: **Dörgicse**: 3.0±0.3 ml/100g, **Szomód**: 3.2±0.3 ml/100g), while significant differences were detected in the accumulation of the essential oil of *L x intermedia* 'Grappenhall' in both experiment years (2017: **Dörgicse**: 5.2±0.2 ml/100g, **Szomód**: 7.3±0.2 ml/100g; 2018: **Dörgicse**: 4.4±0.6 ml/100g, **Szomód:** 7.5±0.7 ml/100g).

While the essential oil content of 'Grosso' did not change significantly according to the production sites, the quality composition of the volatile components of this variety showed the greatest variability: all of the tested components differed significantly in both years. When there was a significant difference in the accumulation of volatile components, the area at Szomód showed the higher essential oil content. We associated this with the drier weather of this production site before flowering and also with the difference between the soil properties of the two production sites. Comparing the two species, the volatile components of the *L. × intermedia* varieties were more significantly affected by the effect of the production site.

Total polyphenol content values also showed significant variability in both species depending on the production site, however, this was less pronounced in the case of the antioxidant capacity values, with the exception of the *L. × intermedia* 'Grosso' variety ($p<0.0001$). In contrast to the essential oil content, the total polyphenol content was higher at the Dörgicse production site. We found that the interaction of the production site and species effects in the total polyphenol content and antioxidant capacity values can be verified statistically (production site*variety: TPC: $p<0.001$; FRAP: $p<0.001$), as was the case with the previously examined factors.

4 ASSESSMENT OF THE RESULTS

In addition to the difference known in the content and composition of the essential oil of lavender species, we found that essential oil content is significantly dependent on the variety (genotype). This is also referred to in previously published literature, albeit in a much narrower context (Muñoz-Bertomeu et al., 2007). Although several varieties show a high degree of variability depending on the production site and year, according to our results, the essential oil content did not change significantly in the samples of the true lavender varieties 'Aromatico Silver', Maillette', and 'Munstead' of different origin, thus the stability of these varieties is favourable.

In true lavender varieties, significantly higher essential oil content values were detected in the stage after flowering than in the full flowering stage in both studied years (2018, 2019). This finding contradicts several previously published data (Boeckelmann, 2008; Cantor et al., 2018). *L. × intermedia* varieties showed a higher essential oil content during the full flowering stage than before or after, which is consistent with the results of Boeckelmann (2008) and Baydar and Erbas (2009). It can be stated that the effect of phenophase on the accumulation of volatile components is mainly characteristic of the lavender species, although some minor differences may also occur in dynamics between varieties.

In our experiment, the percentages of linalool, trans-β-ocimene, terpinene-4-ol, α-terpineol, and lavandulol increased in both years as flowering progressed, which is consistent with the statements of Guitton et al. (2010) and Cantor (2018). According to a study by Guitton et al. (2010), the highest proportions of linalool, terpinene-4, 1.8-cineol, ocimene, and limonene can be found in the flowering and after flowering stages of lavender, which may play a presumably repellent role in the protection of generative organs (Lane et Mahmoud, 2008). Boeckelmann (2008) found that in true and hybrid lavender ('Munstead' and 'Grosso'), the percentage of linalool increased as the flowering stage progressed, which is also consistent with our own research results. However, the highest linalyl acetate value was measured in full flowering in most of the samples, which later decreased in the case of most varieties. This statement is consistent with what was described by Guitton et al. (2010) in their experiment. According to the hypothesis of Raguso et Pichersky (1999) and Schiestl et al. (2001), linalyl acetate and many sesquiterpenes are involved in pollination as attractive molecules, which may explain the maximum at full flowering.

Considering sesquiterpenes in our experiment, caryophyllene oxide and β-caryophyllene steadily decreased as flowering progressed, the former specifically in 2019, the latter mainly in 2018. As highlighted by other authors, in other species the production of β-caryophylene, for example, plays a role in fighting against herbivores (Huang et al., 2013) and pathogens (Sabulal et al., 2006).

In our experiments, we found a significant difference between the production sites in relation to the essential oil content of lavender varieties. Experimental data of Burillo (2003) are consistent with our results as significant differences were found in the samples of *L. latifolia* taken from different Aragonese production sites considering essential oil content.

In terms of production year, true lavender varieties typically accumulated more essential oils (with 1.4-2.8 ml/100g DW) at Soroksár in 2018 compared to 2019 ('Budakalászi': $p<0.018$; 'Munstead': $p<0.0001$; 'Maillette': $p<0.0001$). Among the hybrid lavender varieties, 'Judit' and 'Grappenhall' showed significant differences in the accumulation of essential oil in different years. The higher values were also measured in 2018 ('Grappenhall': $p<0.034$; 'Judit': $p<0.004$). The reason for the difference was presumably that in 2019 significant amounts of precipitation fell in the two weeks preceding the sampling date of both lavender species (true lavender varieties: 13th June, hybrid varieties: 3rd July), which probably had a negative impact on the accumulation of essential oil in the lavenders. In contrast, regular but much smaller amounts of precipitation were characteristic during flowering in 2018.

Based on the results of our experiments and the analysis of previous literature data, we found that a negative correlation can be assumed between the amount of precipitation and the essential oil content of lavender (Yahia et al., 2019). However, it is clear that the absolute quantities of water supply also play a role in such comparisons, and the precipitation conditions of the growing site, together with other parameters, affect the concentration of volatile compounds.

In our experiment, presumably due to the drier production site at Szomód, the stocks of the same varieties had higher linalyl acetate and 1.8-cineol content in their essential oils in both years than those at the Dörgicse site. The proportion of the above components is usually higher in drier areas (Fernández-Sestelo et Carrillo 2020). Other authors also emphasize the negative correlation of the accumulation of oxidized monoterpenes (linalool, terpinen-4-ol, α-terpineol, borneol, camphor, 1.8-cineol) with the amount of precipitation (Yahia et al., 2019).

In summary, we can conclude that the accumulation of essential oil of lavender species is more significantly affected by the weather of the given year, especially the amount and distribution of precipitation, than the composition of the essential oil. The latter may also change depending on the conditions, even significantly, but clear trends cannot be established on the basis of our results. This may be the result of the fact that the biosynthesis of terpenoid volatile components is likely to be much more complex than thought previously, the individual participating enzymes have multifunctional properties, they can produce several end products depending on the conditions and precursors, and the formation of many terpenoid components can be catalysed by several enzymes (Demissie et al., 2012). Thus, not only the environmental sensitivity of the particular enzymes, but also their interactions affect the essential oil spectrum.

Significant differences were found between the two species of lavender in terms of both antioxidant capacity ($p<0.0001$) and total polyphenol content ($p<0.0001$), clearly in favour of the hybrid lavender, which contradicts the results of the experiments of Blažeković et al. (2010). In our work, the 'Grosso' variety is most sensitive to external conditions, while 'Grappenhall' and *L. angustifolia* 'Munstead' are more stable in this respect.

In our experiment, the effect of ontogenesis on the total polyphenol content and antioxidant capacity occurred in the case of true lavender and showed different tendencies for each variety, on the contrary it had no significant effect on the phenolic compounds of hybrid lavender varieties. In the case of TPC values, the interaction of the species factor and the flowering stage factor was verified statistically as well ($p<0.005$). We also proved the interaction of the flowering stage and the varieties ($p<0.001$), but we were unable to provide a definite trend in relation to the development phases for either parameter.

In our experiment, we proved the effect of organ diversity on the phenolic and antioxidant compounds. This effect was significant in the case of hybrid lavender varieties and less significant in the case of true lavender. We found that in all cases, higher phenol concentrations were detected in the inflorescences during the main flowering in the summer (the difference is 103.5-152.5 mg GAE/g DW). This statement contradicts the study of Blažeković et al. (2010), but is consistent with the experiment of Nurzyńska-Wierdak et Zawiślak (2016).

Although the higher values in our samples collected during the second flowering period in autumn were mostly measured in the leaves, the differences between the organs are not significantly manifested in all sample pairs. Phenolic compounds are found in higher concentrations in the leaves of the varieties we examined than the literature data indicates, 59.1-253.2 mg GAE/g DW in *L. angustifolia* samples and 95.3-308.6 mg GAE/g DW in *L.* × *intermedia* samples. Bouayed et al. (2007) detected only 16.2 mg GAE/g DW total phenol content in the leaf of *L. angustifolia*. Based on the above, the majority of literature data are in line with our data in the second flowering. It can be presumed that the distribution of the quantity of polyphenols by organs also depends on changes in seasons and weather conditions. It is known that the plant's defence mechanism is active in the plant organ where it is needed according to the biorhythm. Thus, in the autumn period, the plant is likely to protect its vegetative organs (leaves) in order to prepare for the cold winter period and to facilitate overwinter (Parejo et al., 2001). Organ differences would be particularly important if the amount of phenoloid compounds in lavender is prioritised in product development in the future.

Our studies confirm that the time of sampling (seasonal) also influences the concentration of polyphenols and antioxidant compounds, in line with previous findings by some authors (e.g., Brasileiro et al., 2015). In our case, significantly higher values were characteristic of the October cut. Based on previous literature data, we assume that higher autumn values may be related to higher rainfall and cooler autumn climates, as stress responses, in the case of lavender with Mediterranean origin. The effect of abiotic effects on the accumulation of phenoloids is probably the result of even more complex processes than described in the case of volatile terpenoid components. The phenomenon and importance of "stress" may depend on several factors, therefore the comparison of previous publications is also limited. In addition to the weather conditions of the given year, the synthesis of phenoloids can be influenced by other conditions of the production site, and the effect may even vary in each species and variety.

According to our results, contrary to the accumulation of essential oil content, the total phenol content and the compounds with antioxidant capacity accumulated in higher concentrations at the Dörgicse production site. Since it is known that the production of phenolic compounds is triggered earlier by the plant in response to stress (Goodger et al. 2013), we also examined possible stress factors in this respect. Although the $CaCO_3$ content (by 6 m/m) was lower and the macro- and microelement values (mg 248 mg/kg, Na 78 mg/kg and Mn 188 mg/kg) were higher than at the site at Szomód, their assessment as stress would require further studies. Our experiment proves that there is an interaction between the effects of production sites and the variety effects: region*variety: TPC: $p<0.001$; FRAP: $p<0.001$, i.e., genotype, as with other factors discussed previously, influences the response to environmental effects.

5. NEW SCIENTIFIC RESULTS

1. Diversity of lavender species and varieties

Based on our studies, we were the first to find that the intraspecific difference between *L. × intermedia* varieties regarding the composition of essential oil was more significant than in the case of *L. angustifolia* varieties. Among the varieties studied, the linalool proportion of 'Maillette' and 'Grosso' are outstanding compared to all previous data (55-58%).

By comparing the two species, we stated that hybrid lavender has significantly higher TPC and FRAP values than true lavender and differences in the varieties are also smaller in *L. × intemrdia*.

2. Interactions of the environment and the taxon

We were the first to verify that the lavender species and varieties have a strong significant interaction with production site conditions, harvest time and year of production, i.e., they show different variability and stability with differences in the environmental factors. We found that there were more stable, less variable taxa ('Munstead' and 'Grappenhall') and varieties that responded strongly to the year and production site effects ('Beate', 'Grosso') among the varieties of both species.

The effect of production year and the production site was detected in all cases in terms of TPC and FRAP values, while production year affected the essential oil content in only one of the production sites.

The effect of production year had a stronger influence on the essential oil components of the *L.* × *intermedia* species than on that of the *L. angustifolia* species (e.g., 1.8-cineol, linalool, camphor, α-terpineol, linalyl acetate).

3. Effects of the phenophase and the organ composition on the compositional values of lavender

We were the first to prove that during the flowering phase, the time period of the maximum accumulation of essential oils and that of the composition corresponding to the pharmacopoeia/standard are different in the case of the two species. To achieve the highest essential oil content, the optimal harvest time of true lavender is the stage after flowering, while that of hybrid lavender is full flowering. However, the proportion of linalyl acetate, together with the total ester percentage decreases after flowering, therefore harvesting in full flowering may also be favourable in the case of this species as well.

We also verified that, compared to the characteristics of the essential oil, the flowering stage has less influence on the total polyphenol content of lavender varieties. Among true lavender varieties, the highest TPC and FRAP values can be measured in the budding stage, while in the case of hybrid lavender, the highest TPC can be detected after the flowering stage, but the maximum antioxidant capacity can be detected during the full flowering stage.

The effect of organ diversity on the compounds with phenolic and antioxidant effects was more significant in the case of hybrid lavender varieties. However, the level of accumulation in inflorescences and leaves depends on the cutting period as well.

4. Variability of compositional parameters

We proved that the most stable essential oil components of the studied lavender taxa are β-myrcene, lavandulol, α-terpineol and geranyl acetate. Significant differences in these essential oil components regarding the variety, production year and production site are minimum. At the same time, terpenoids with significant differences most often are linalool, terpinene-4-ol, and caryophyllene oxide.

The main components (linalool, linalyl acetate) were fundamentally influenced significantly by the variety and phenophase within the species. In addition, year and production site had an impact on their share within the essential oil, and these changes were most pronounced in hybrid lavender varieties.

The most important factors influencing the total polyphenol content and antioxidant capacity values in lavender were genotype (variety), organ diversity, production year and harvest time.

Results that have practical use

1. We characterised the available essential oil content and quality together with the total polyphenol content and antioxidant capacity values *of L. angustifolia* 'Aromatico Silver', 'Budakalászi', 'Beate', 'Hidcote', 'Maillette', and 'Munstead', as well as those of *the L. x intermedia* varieties 'Grosso', 'Grappenhall', and 'Judit' under the production site conditions in Hungary. We have identified the factors that most influence drug quality.
2. It was found that considering the studied *L. angustifolia* varieties, the essential oil content only of the 'Hidcote' stock at Dörgicse complied with the requirements of the seventh edition of The Hungarian Pharmacopoeia and the tenth edition of the European Pharmacopoeia in both experimental years except for the values of α-terpineol. The amount of essential oil in all studied varieties at Dörgicse far exceeded the 1.3 ml/100 g DW value specified for *Lavandulae flos* in the pharmacopoeia article.
3. Based on our experimental results, we recommend, from the studied varieties, *L. angustifolia* 'Hidcote' and *L. × intermedia* 'Grosso' varieties to the Hungarian producers. These two varieties can be highlighted for the quality of their essential oil, while 'Munstead' can be highlighted for the stability of the properties of the essential oil. If the target is high total polyphenol content, we recommend harvesting in the autumn, in October, either from the leaves of the hybrid variety 'Grappenhall' (TPC: 311.7 mg GAE/g DW) or from the inflorescences of 'Grosso' (FRAP: 287.7 mg AAE/g DW). Considering the summer period, the green bud stage *of L. angustifolia* 'Budakalászi' can be highlighted due to its high total polyphenol content (TPC: 365.1 GAE/g DW).
4. Our experiments prove that the *L. × intermedia* species is more sensitive to abiotic factors (production year effect, production site effect) than *L. angustifolia* in terms of essential oil properties. In addition, the effect of the genotype (variety) on volatile components, as well as the effect of organ diversity on all polyphenol content and antioxidant capacity, were found to be more significant in the hybrid lavender species.

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7. LIST OF PUBLICATIONS RELATED TO THE DOCTORAL THESES

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