

Theses of PhD dissertation

**STUDY FOR THE FERTILIZER
RESPONSES OF MEDICINAL PLANTS IN
DIFFERENT CROPYEARS**

Judit Éva Lelesz

supervisor:
Dr. József Csajbók
associate professor



UNIVERSITY OF DEBRECEN

**Kálmán Kerpely Doctoral School of Crop Production, Horticulture
and Regional Sciences**

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1. INTRODUCTION

By the beginning of the 21st century, the increase in the turnover of herbal medicinal products will outweigh the expansion of the use of medicinal products. According to international market forecasts, the proportion of medicinal, health preserving, nutritional supplement and other special purpose products is expected to increase.

The large growth of herbal production in developing countries and its appearance on the international market poses new challenges for Hungarian herb growers. No comprehensive research on the nutritional requirements of herbs has been carried out so far, however, because of the increased market demand, it would be beneficial to provide more information to the growers.

Under my research I investigated the marigold's (*Calendula officinalis* L.), the summer savory's (*Satureja hortensis* L.) and the purple coneflower's (*Echinacea purpurea* L.) nutrient requirements with different nutrient supply settings, in small plot trial, with particular regard to the quantity of the drug, the composition and distribution of the volatile substances in the drug.

2. MATERIAL AND METHODS

I set up my experiment in the experimental garden of the Agricultural Research Institutes in the University of Debrecen. The soil of the experiment was chernozem. Plot size was 8 m² and plots were arranged in 4 replications in randomized blocks, with 6 different fertilizer treatment levels. The fertilizer doses were from N 15 kg ha⁻¹, P₂O₅ 20 kg ha⁻¹ and K₂O 30 kg ha⁻¹ to N 75 kg ha⁻¹, P₂O₅ 100 kg ha⁻¹ and K₂O 150 kg ha⁻¹ in 5 levels with 15 kg ha⁻¹ N, 20 kg ha⁻¹ P₂O₅, and 30 kg ha⁻¹ K₂O steps. Nutrients were not applied to the control plots. The sowing of the marigold and summer savory took place on the spot in 4 rows with 40 cm row space, in 1 cm depth every year in April. In the case of purple coneflower I used planting.

The height of the plants was measured with a tape measure in 2015 and 2016 for four plants per plot, five in 2017 for marigold and summer savory and ten for coneflower in 2016, 2017 and 2018.

SPAD values measurements of the marigold were carried out in 2017 and 2018 on 15 and 10 plants on a Konica Minolta Chlorophyll Meter SPAD-502 Plus. **Savory's NDVI (Normalized Difference Vegetation Index) measurements** were three times in 2017 with Trimble GreensSeeker Handheld Crop Sensor (Model Number: HCS-100). Dragging the instrument over the plots and holding the measuring knob down gave me an average value for that plot. In 2018, the same instrument and measurement method was used to examine the coneflower also.

The marigold flower drug was gathered several times a year, with one centimeter long stem, from an inner row of each parcel. I

measured the raw drug, then it was spread in one layer, in plastic crates and dried in half shade.

I harvested the savory's herb once a year. I cut the herb one inch above the ground and then measured the raw weight. For drying and calculation of drying loss, a sample of known weight per plot was taken, which was dried in a drying oven at 40 C° and then were crushed. I calculated the loss of crushed drug production based on the percentage relationship between raw and crushed drug production.

The flowering herb of the coneflower was gathered with 25-30 cm long stem annually from an inner row of each parcel. I measured the raw weight, then took a sample for drying and calculating the drying loss. In 2016, samples were dried in one layer, in plastic crates under half shade for two weeks. In 2017 and 2018, I dried it on 40 C° in the drying cabinet because of the rainy weather. The dried drug was stored in paper bags and lockable plastic bags for all three plants. On the 7th of November in 2017, I took root samples from the coneflower, 20 per plot. I measured the raw weight, then took samples for the volatile matter measurements, and for drying, which was in drying oven, on 40 C°.

Volatile material identification was carried out on the sample of dry drug of all three plants and raw root drug of the coneflower. SPME-GC / MS method was used.

GC-MS analyzes were performed on a Hewlett-Packard 5890 Series II gas chromatograph - 5971A mass spectrometer with the following analytical context:

- Column: HP-5 stationary phase capillary column (25 m x 0.25 mm x 0.25 μ m)
- Carrier gas: helium (1 mL/min, 40 C $^{\circ}$), constant injector pressure
- Analytical temperature: 50 C $^{\circ}$ for 2 minutes, 20 C $^{\circ}$ /min increase to 150 C $^{\circ}$ increase 15 $^{\circ}$ C / min to 240 $^{\circ}$ C (10 min)
- Total Analysis Time: 23 minutes
- Injector temperature: 200 C $^{\circ}$
- Injector liner: uncharged silanized liner
- Transfer line temperature: 280 C $^{\circ}$
- Ionization: 70 eV
- Weight Range: 10-500 AMU

The gas chromatographic mass spectrometer control, data acquisition and evaluation were performed with Hewlett-Packard GC-MS Chemstation rev.3 software. The components were identified using mass spectra and Nist98 databases. During data processing I performed variance analysis and Pearson correlation analysis using MS Excel 2010 and IBM SPSS 22.0 software packages.

3. RESULTS AND DISCUSSION

3.1. RESULTS OF THE MARIGOLD'S AGRONOMIC MEASUREMENTS

The $N_{30}P_{40}K_{60}$ and the $N_{45}P_{60}K_{90}$, $N_{60}P_{80}K_{120}$ treatments had the highest plant height values, although this was not supported by the variance and Pearson correlation analyzes. Several effects have been detected between the meteorological factors and the height of the marigold. In all cases, the temperature, the soil temperature and the global radiation had a positive correlation with the plant's height. In the short term (1 week) the temperature of the soil has the greatest effect, followed by the average temperature. The effect of the global radiation on a one-week interval is not statistically measurable, but the more time we take as a basis for examination, its influence increases, until it reaches its strongest correlation value. In the eight week interval, this correlation relationship is stronger than the average temperature's, but not stronger than the soil temperature's.

The precipitation has a similar positive relationship with the plant's height in a shorter period of time, while a negative influence of the air humidity was detected. It follows that a longer, humid period's effect is double for the plant's height increase. On the one hand, at intervals of five weeks or less, the amount of precipitation has a positive effect, on the other hand, if this period is extended, the growth of the plant may be slowed down by the increased air humidity.

The highest SPAD values in calendula were found in the treatments N₄₅P₆₀K₉₀, N₆₀P₈₀K₁₂₀ and N₇₅P₁₀₀K₁₅₀, which were confirmed by the results of the analysis of variance.

In the joint analysis of the data series of the two investigated years, the correlation relationships are all loose, and in many cases their change, strengthening or weakening is also possible. It can not be stated with absolute certainty that the observed meteorological factors have a significant effect on the SPAD values of the plant.

3.2. RESULTS OF THE SUMMER SAVORIE'S AGRONOMIC MEASUREMENTS

The values of the treatments N₁₅P₂₀K₃₀, N₃₀P₄₀K₆₀, N₆₀P₈₀K₁₂₀ were the highest, although this was not supported by the variance and Pearson correlation analyzes. Examining the three-year data together, the positive correlation of temperature, soil temperature, precipitation, and global radiation with the height of summer savory was confirmed. The temperature and soil temperature has a clear effect both in the short term and in the long term, that is, these two factors influence the height of the plant throughout the growing season. The effect of the precipitation in the one week interval is not yet statistically evaluated, but its effect is steadily increasing. So the right amount of precipitation, even if not immediately, like temperature factors, but in a long term, definitely has an important effect. The effect of air humidity is negative, as in the case of marigold. This effect is not statistically measurable in one, two and three weeks' interval, but then it is steadily increasing. It was most

strongly correlated with the plant's height at seven-week interval, but it was still below the effects of other meteorological factors.

The values of treatments $N_{30}P_{40}K_{60}$, $N_{60}P_{80}K_{120}$ and $N_{45}P_{60}K_{90}$ were the most favorable when measuring NDVI of savory. This was not supported by the results of the variance and Pearson correlation study and only in a few cases, was statistically significant in correlation to the meteorological factors.

3.3. RESULTS OF THE PURPLE CONEFLOWER'S AGRONOMIC MEASUREMENTS

The values of the treatments $N_{30}P_{40}K_{60}$, $N_{45}P_{60}K_{90}$, $N_{60}P_{80}K_{120}$ were the highest, although this was not supported by the variance and Pearson correlation analyzes. If the shortest period (one week) is evaluated, the changes in soil temperature and temperature has the strongest correlation with the height of the coneflower, followed by the correlation of the air humidity and precipitation. Analyzing the correlations at the eight-week interval, the plant was most affected by global radiation, soil temperature, and temperature positively, followed by the negative effect of air humidity. During the three week term, when the effect of precipitation is stronger, it is preceded only by the soil temperature's correlation. All in all, the relationship of soil temperature, temperature and global radiation is crucial for increasing coneflower's height, but the precipitation and air humidity also have a significant effect at certain times of the year.

The NDVI measurements of the purple coneflower showed the highest values for the $N_{45}P_{60}K_{90}$, $N_{60}P_{80}K_{120}$ and $N_{75}P_{100}K_{150}$ treatments. When analyzing the data, we found no correlation

between the values and the nutrient settings in either the variance or the Pearson correlation analysis. Global radiation was negative first, then positive, air humidity first positive, and then negatively correlated with NDVI values. If the one week interval is taken into consideration, the soil temperature, air humidity and precipitation are in positive correlation relationship with the coneflower's NDVI values, while global radiation has a negative effect. During the eight-week period, when the influence of precipitation disappears, air humidity has an as strong negative effect as the temperature, soil temperature and global radiation is positive. The negative effect of air humidity appears again as a risk factor with its negative correlation.

3.4. RESULTS OF THE MARIGOLD'S DRUG AND VOLATILE MATTER INTENSITY

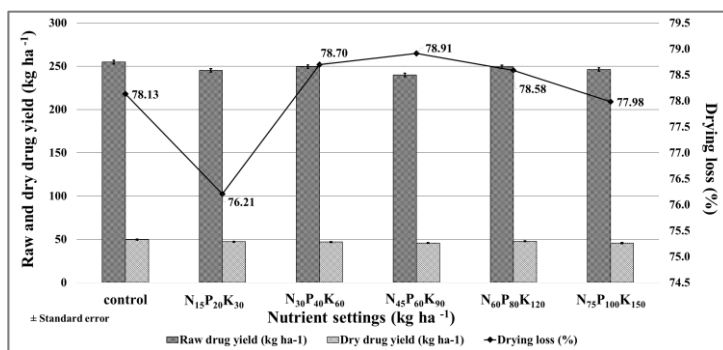


Figure 1 Effect of nutrient treatments on investigated drug yield factors of marigold (Debrecen, 2015-2017)

N₁₅P₂₀K₃₀ nutrient treatment could be beneficial for marigold drug on calcareous chernozem soil. The results show that the higher the

amount of nutrient supplies, the drug's moisture content increases (Figure 1). Increasing the number of harvests has a negative effect on the yield of raw and dry drug, but not on the drying loss. According to Pearson's correlation analysis, the temperature, soil temperature, and global radiation have a negative correlation with raw and dry drug yields and drying loss. As a result of these factors, the plant produces less drugs, but it also has a lower moisture content too. The air humidity and the precipitation has a positive effect on the amount of raw and dry drug, as well as on the drying loss, that is, it increases the drug yield with its moisture content.

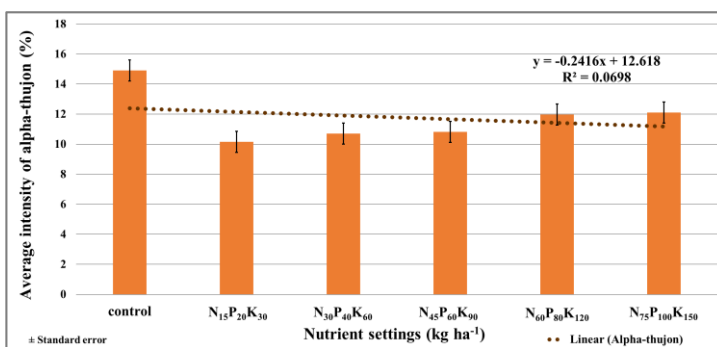


Figure 2 Effect of nutrient treatments on the intensity of alpha-thujon (Debrecen, 2015-2017)

Nutrient treatments had an effect on the drug's volatile composition. As a result of increasing nutrient supplies, alpha-thujon (Figure 2) and germacrene D are decreased while alpha-caryophyllene is increased. These materials are in medium correlation with each other, that is, they can significantly influence the increase or decrease in each other's intensity. Temperature, soil temperature and global

radiation has a positive while the precipitation and air humidity has a negative correlation with volatile matter concentrations, both individually and collectively, but the strength of the correlations' is different.

3.5. RESULTS OF THE SUMMER SAVORIES' DRUG AND VOLATILE MATTER INTENSITY

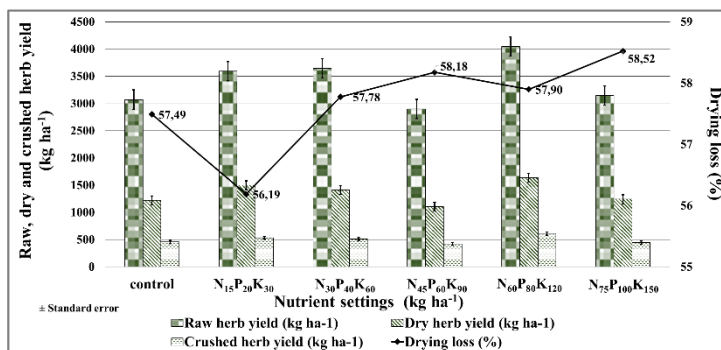


Figure 3 Effect of nutrient treatments on investigated drug yield factors of summer savory (Debrecen, 2015-2017)

N₃₀P₄₀K₆₀ nutrient treatment could be beneficial for summer savories drug yield under similar growing conditions (Figure 3). Higher amounts of nutrients increase the moisture content of the drug and the proportion of the stem in the herb.

Bigger nutrient treatments are beneficial for plant growth and herb yield, but less for the crushed drugs, because they increase drying loss and stem ratio. With the increase of the raw yield, the drying loss increases lesser than the crushed drug production loss, so it is not

necessarily the moisture content that grows, but the ratio of the stem (Figure 4).

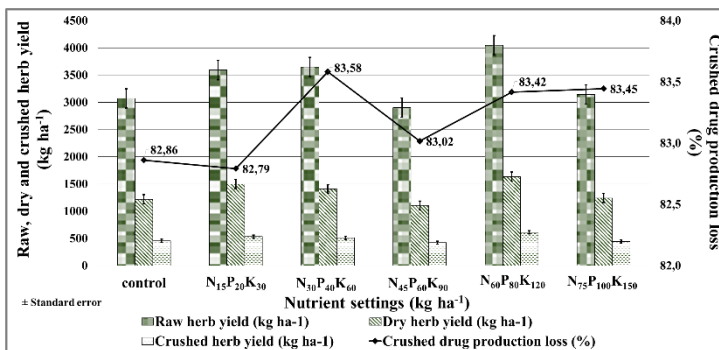


Figure 4 Effect of nutrient treatments on investigated drug yield factors of summer savory (Debrecen, 2015-2017)

The amount of raw, dry, crushed drug and the crushed drug production loss are both negatively influenced by temperature and soil temperature. Drying loss increases with temperature and global radiation, while herb drug yield decrease with its stem ratio. The positive correlation between precipitation and air humidity with all measured yield parameters confirms the assumption that their yield-increasing effect is not an increase in the moisture content of the plant. Global radiation had a negative effect on the raw, dry yield and crushed drug production loss during the 1 week period, at the time of harvest, which coincides with the time of flowering.

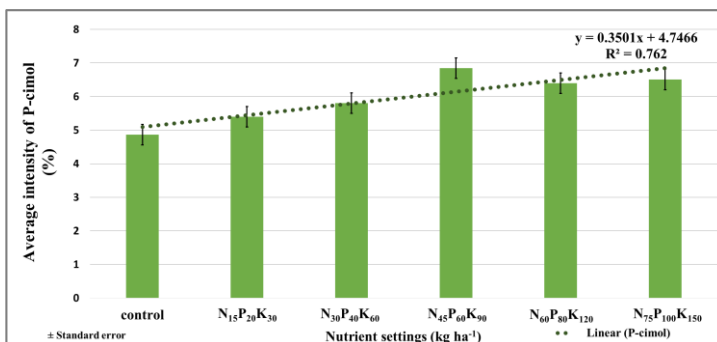


Figure 5 Effect of nutrient treatments on the intensity of P-cimol (Debrecen, 2015-2017)

Nutrient treatments had an effect on the total and individual intensities of the volatile substances of the summer savory. Increasing amounts of NPK fertilizers increase the intensity of P-cimol (Figure 5) while decreasing carvacrol and beta-caryophyllene. These substances are in close correlation with each other, that is the increase in the intensity of P-cymol may be significantly influenced by the decrease of the other two substances. It is true for both the total volatile substance content of the drug yield and the intensity of the investigated individual substances that they are most influenced (negatively) by temperature, soil temperature and global radiation in the short term, ahead the precipitation and air humidity with positive effects. This order is reversed in long term.

3.6. RESULTS OF THE PURPLE CONEFLOWER'S DRUG AND VOLATILE MATTER INTENSITY

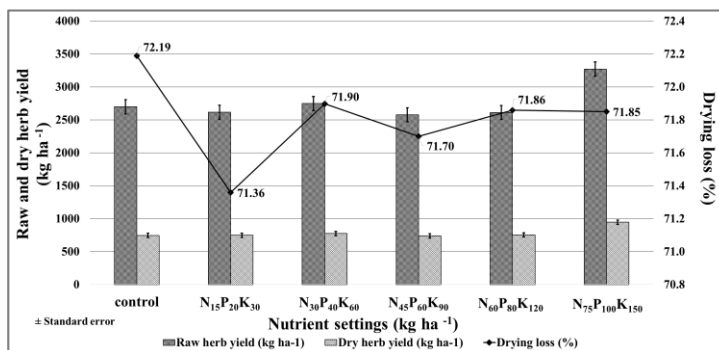


Figure 6 Effect of nutrient treatments on investigated herb drug yield factors of purple coneflower (Debrecen, 2016-2018)

Based on the results of the experiment, for the cultivation of herb drug of the purple coneflower, the N₄₅P₆₀K₉₀, for the root the N₇₅P₁₀₀K₁₅₀ nutrient treatments are advantageous on calcareous chernozem soil (Figure 6).

According to Pearson's correlation analysis, the temperature "increases" the drying loss, but not the raw and dry crop, that is the moisture content of the herb increases. Precipitation and air humidity also increase the yield's moisture content, but are likely to inhibit flower production, thereby reducing the yield. The increase of soil temperature increases drying loss in the short term, but decreases in the long term while increasing raw and dry yield that is the dry matter content of the herb increasing. The effect of global radiation is not always clearly evident in drying loss, but when it does, it reduces it and increases the yield.

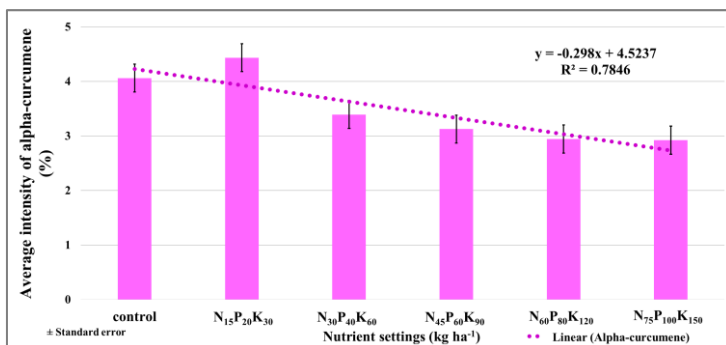


Figure 7 Effect of nutrient treatments on the intensity of alfa-curcumene in the coneflower herb (Debrecen, 2016-2018)

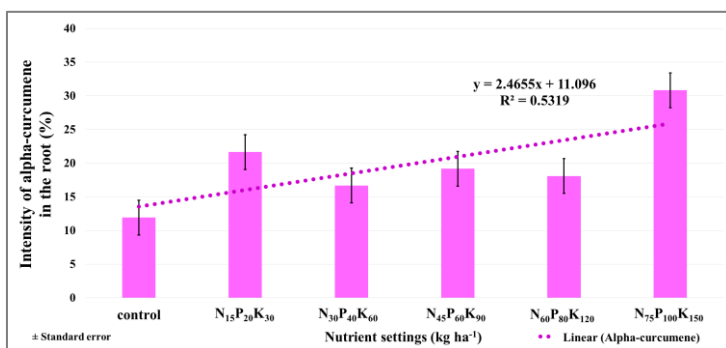


Figure 8 Effect of nutrient treatments on the intensity of alfa-curcumene in the coneflower root (Debrecen, 2017)

The total and individual intensity of the volatile materials of the herb and root drug of the coneflower were influenced by the nutrient treatments. Increasing amounts of NPK fertilizer decreased the intensity of alpha-curcumene (Figure 7), germacrene D and gamma-murolene in the herb drug of the purple coneflower. Increasing amounts of NPK fertilizer increased the intensity of alpha-curcumene

(Figure 8), and P-cimen, and the thymol's closed with the $N_{30}P_{40}K_{60}$ treatment in the root.

4. NEW SCIENTIFIC RESULTS

1. Increased doses of NPK fertilizers had a positive effect on the height of marigold, summer savory and purple coneflower and on the NDVI values of savory and coneflower and SPAD values of marigold. $N_{30}P_{40}K_{60}$ treatment resulted in highest plants in marigold and summer savory, while $N_{45}P_{60}K_{90}$ was the best treatment for coneflower. Marigold SPAD values were increased the most by $N_{45}P_{60}K_{90}$, savory NDVI values were increased by $N_{30}P_{40}K_{60}$, and coneflower NDVI values by $N_{45}P_{60}K_{90}$.
2. An increase in drug yield was observed as a result of nutrient treatments for all three plants. The most favorable nutrient settings were $N_{15}P_{20}K_{30}$ for marigold, $N_{30}P_{40}K_{60}$ for summer savory, $N_{45}P_{60}K_{90}$ for coneflower's herb and $N_{75}P_{100}K_{150}$ for its root. As a result of increasing nutrient treatments, the drying loss of drug yields - and the crushed drug production loss of summer savory - also began to increase, with the exception of the root drug of the coneflower. Multiple harvesting decreased the raw (up to 80 %) and dry weight (up to 84 %) of the marigold drug yield, but did not change its drying loss (26%).
3. The effect of the meteorological factors examined during the experiment is different in sign and strength with respect to the drug yield and volatile parameters of the examined plants. Increases in temperature (marigold $r = -0.520$, summer savory $r = -0.678$), soil temperature (marigold $r = -0.488$, summer

savory $r = -0.729$), and global radiation (marigold $r = -0.429$) reduced the drug production of marigold and summer savory. There was a positive relationship between the volatile matters of marigold and the temperature ($r = 0.490$), the soil temperature ($r = 0.489$) and the global radiation ($r = 0.490$). In case of the summer savory the temperature ($r = -0.356$), the soil temperature ($r = -0.345$), and global radiation ($r = -0.354$) had a negative effect. In the case of the coneflower, the rising temperature increased the drying loss ($r = 0.656$), but did not affect the raw ($r = 0.224$) and dry drug ($r = 0.122$) yields. Precipitation ($r = 0.878$) and air humidity ($r = 0.725$) also increased moisture content but decreased the herb yield.

4. The total and individual volatile matter intensities were influenced by the nutrient settings and examined the meteorological factors. Increasing amounts of NPK fertilizer treatments reduced the intensity of alpha-thujon (from 14.9 % to 10.15 %) and germacrene D (from 9.31 % to 6.26 %) in marigold inflorescences while increase alpha-caryophyllene (from 2.24 % to 4.15 %). As a result of nutrient treatments in the summer savory herb, P-cimol's intensity increased (from 4.87 % to 6.85 %) while carvacrol (from 77.95 % to 72.01 %) and beta-caryophyllene (from 0.71 % to 0.46 %) decreased. In the coneflower herb, the intensity of alpha-curcumene (from 4.44 % to 2.92 %), germacrene D (from 16.26 % to 11.27 %) and gamma-murolene (from 2.78 % to 2.19 %) decreased in the treatments, while in the root the intensity of alpha-

curcumene (from 11.92 % to 30.82 %), P-cymene (from 1.44 % to 3.85 %) and thymol (from 0.38 % to 17.62 %) increased (up to N₃₀P₄₀K₆₀ treatment) increased.

5. The individually investigated volatile matters intensities also affect each other. In the marigold drug, there was a moderate correlation of alpha-thujon with alpha-caryophyllene ($r=0.619$) and germacrene D ($r=0.614$), and alpha-caryophyllene with germacrene D ($r=0.727$), on 1% level of significance. In the summer savory herb drug, P-cimol was highly correlated with carvacrol ($r=0.926$) and beta-caryophyllene ($r=0.866$), and with carvacrol with beta-caryophyllene ($r=0.953$).

5. PRACTICAL RESULTS

From the point of view of marigold drug production, the $N_{15}P_{20}K_{30}$ nutrient setting could be advantageous on calcareous chernozem soil. The results show that the higher the nutrient used, the higher the moisture content of the drug.

From the point of view of summer savory herb drug production, the $N_{30}P_{40}K_{60}$ nutrient setting may be advantageous under growing conditions similar to the experiment. Excessive application of nutrients increases the moisture content and the ratio of stems in the herb, which increases the cost of primary processing (drying and crumbling) and reduces the amount of marketable crushed drug.

According to the results of the experiment, the nutrient treatment of $N_{45}P_{60}K_{90}$ is advantageous for calcareous chernozem soil in terms of cultivation of purple coneflower herb and root. I think it is worth harvesting the herba even if the main purpose of cultivation is the root drug.

My reasoning is as follows:

1. By harvesting the flowering herb, we relieve the plant of its crop production, meaning that it can use its energy to increase its vegetative organs.
2. Although the plant consumes energy for the flowering herb, because of the date of full flowering, the plant is not characterized by further inflorescence production during the rest of the vegetation period.
3. The market value of the harvested herba also justifies its harvest, so we make the plant "dual-use". In this case, it is

advisable to apply a larger amount of nutrients (N₆₀P₈₀K₁₂₀ or even N₇₅P₁₀₀K₁₅₀ at setting) so that after harvesting the herb, the plant does not regenerate at the expense of the root growth.

In addition to nutrient settings, the investigated meteorological factors also have a significant influence on the raw and dry drug yields and drying losses, so these should be taken into account in the site characteristics.

The following factors influence the choice of **the appropriate nitrogen, phosphorus, and potassium nutrient application** for each of the three plants:

- maximizing the marketable drug production
- minimize the cost and time factors for primary processing (drying and crumbling)
- soil conditions of the production area
- the climatic conditions of the area

The negative effect of air humidity appeared in the examination of the agronomic and drug production factors of each plant. Increasing the row spacing is a possible agrotechnical solution to counteract the negative impact of air humidity. This also reduces the shading effect of the individual plants. However, due to the lower soil coverage, it may dry out more quickly and the potential negative effects of global radiation may occur with SPAD values. Increased air humidity in the herd may also play a role in the appearance of fungal diseases. As the plant ages, this risk increases. This is evidenced by the appearance of powdery mildew on the marigold in early August in all three years.

The 40 cm row space was chosen because of the size of the experimental area. This is not advantageous for any medicinal plant, since the stock is so dense that it makes it difficult to move and helps to increase the air humidity.

For each of the three investigated plants, it was found that the total and individual volatile matter's intensities are influenced by the nutrient settings. The intensities of the examined volatile matters also have an influence on each other and have a correlation relationship of the same strength as the meteorological factors. This "correlation network" can significantly influence the intensity and composition of volatile substances in the drug. Therefore, it would be worthwhile to conduct a comprehensive study of the relationships and variability of the volatile matter intensity of these plants. If this succeeds, changing the technology of crop production would make it possible to increase or decrease the intensity of certain substances in the drug according to the growing needs.

6. LIST OF PUBLICATIONS



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Registry number: DEENK/273/2019.PL
Subject: PhD Publikációs Lista

Candidate: Judit Éva Lelesz
Neptun ID: C24Q8R
Doctoral School: Kálmán Kerpely Doctoral School
MTMT ID: 10052577

List of publications related to the dissertation

Foreign language scientific articles in Hungarian journals (5)

1. **Lelesz, J. É., Csajbók, J.:** Analysis of different fertilization settings' effect in the case of the summer savory's (*Satureja hortensis* L.) yield and active agents.
Agrártud. Közl. 74, 101-105, 2018. ISSN: 1587-1282.
2. **Lelesz, J. É.:** The purple coneflower's (*Echinacea purpurea* L.) nutrient requirements investigation in a small plot trial.
Agrártud. Közl. 74, 95-99, 2018. ISSN: 1587-1282.
3. **Lelesz, J. É., Csajbók, J.:** The marigold's (*Calendula officinalis* L.) drug yield and economic value changes over time and composition of the essential oil active agents under different fertilization settings.
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DOI: <http://dx.doi.org/10.18380/SZIE.COLUM.2017.4.1.suppl>
4. **Lelesz, J. É., Nagy, É., Csajbók, J.:** The marigold's (*Calendula officinalis* L.) essential oil components and drug yield under different fertilization settings.
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5. **Lelesz, J. É., Nagy, É., Csajbók, J.:** The marigold (*Calendula officinalis* L.) drug essential oil agents change under different fertilization settings in small plot trial.
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Foreign language scientific articles in international journals (1)

6. **Lelesz, J. É.:** Two grown herbs' drug yield changes under different fertilization settings in small plot trial.
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7. **Lelesz, J. É.:** A körömvirág drogtermésének változása az ökológiai tényezők és eltérő trágyakezelések hatására kispárcellás kísérletben.
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8. **Lelesz, J. É., Nagy, É.:** A borsikafű (*Satureja hortensis* L.) drogtermésének és illóolaj hatóanyagainak változása eltérő trágyakezelések hatására kispárcellás kísérletben.
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9. **Lelesz, J. É., Nagy, É.:** A borsikafű (*Satureja hortensis* L.) herbadrog termésének és illóolaj hatóanyagainak változása eltérő trágyakezelések hatására kispárcellás kísérletben.
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10. **Lelesz, J. É., Nagy, É.:** A borsikafű (*Satureja hortensis* L.) tápanyagigényének vizsgálata kispárcellás kísérletben.
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11. **Lelesz, J. É., Nagy, É.:** A körömvirág (*Calendula officinalis* L.) tápanyagigényének vizsgálata kispárcellás kísérletben.
Agrártud. Közl. 68, 61-66, 2016. ISSN: 1587-1282.

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12. **Lelesz, J. É.:** A körömvirág drogtermésének változása az ökológiai tényezők és eltérő trágyakezelések hatására kispárcellás kísérletben.
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13. **Lelesz, J. É., Nagy, É.:** A borsikafű (*Satureja hortensis* L.) drogtermésének és illóolaj hatóanyagainak változása eltérő trágyakezelések hatására kispárcellás kísérletben.
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17. **Lelesz, J. É., Csajbók, J.:** The changes of the purple coneflower's (*Echinacea purpurea* L.) herb and radix drug yield under different fertilization conditions.
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Élet Tud. 72 (49), 1542-1544, 2017. ISSN: 0013-6077.

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