The Purple Coneflower's Herb Yield's and Essential Oil Agents' Change under Different Fertilization Settings

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***Abstract*—During our research we investigated the purple coneflower's (*Echinacea purpurea L.*) herb yield's and it's essential oil active agents' change under different fertilization settings in small-plot trial. We measured the raw drug yield, which we harvested in 2016 and in 2017. We made the harvest and all other works manually. We dried the harvested herba under prenumbra for three weeks. Based on the data, every fertilization settings' yield was less than the control plots' in 2016. In contrary to the 2016 year's data, in 2017 we measured the highest yield data in the N75P100K150 fertilization setting. The drying loss of the investigated two years' herb yields' were fluctuating between the nutrient requirements.**

We made single-factor variance analysis, Pearsons's correlation test and Factor analysis to investigate the connection between the quantity of the herba yield and the different nutrient settings. We discovered a complex and complicated connection system between the different essential oil active agents. We used SPME (Solid Phase MicroExtraction) and GC-MS (Gas Chromatograph-Mass Spectrometer) we examined the effects of the different fertilization settings for the herb's main active ingredients of essential oil's percentage.

***Index Terms*—herb, medicinal plant, coneflower, nutrient requirement, fertilization**

I. Introduction

The cultivation and use of medicinal plants nowadays is a re-discovered research field. The phytotherapy is getting more emphasis again in traditional medicine [1]. There is an increasing need to develop methods of nutrient supply that ensure profitable yields the directives of environmental protection [2].

The purple coneflower is an ancient medicinal plant of the United States' meadows and prairies [3]. Three species and many hybrid varieties – ornamental plants – known [4].

In traditional and modern medicine, the *E. purpurea* (spread in wet climatic hilly areas), the *E. angustifolia* and the *E. pallida* the typical plants of the prairie used. Its breeded variety in Hungary the "Indián" was made specially for medicinal use [4].

The purple coneflower could be reproducible with sowing on place, or division, or seedling. It's cultivation is 2-3, or 4 years long [4].

The purple coneflower is nutritious and lime-consuming. It is best grown in medium-sized, well-water-rich, humus and nitrogen-rich chernozem soils. The well-nutrient, humus-rich sandy soils are also suitable for the production of root drugs. Considering the nutritional properties of the soil, filling fertilization is required before planting. Under or below the foreground plant the application of large amounts of organic fertilizer (30 t * ha^{-1}) and nitrogen fertilization during the vegetation period is necessary [4].

The *Echinacea* genus is allogam, and it's populations are genetically heterogeneous [5].

The drug of these plants is the herba (*Echinaceae purpureae herba, Echinaceae angustifoliae herba, Echinaceae pallidae herba*), and the root (*Echinaceae purpureae radix, Echinaceae angustifoliae radix, Echinacea pallidae radix*) [6].

It can be used as prevention or supplementary treatment in many different diseases. It could be useful for the supplementary treatment of burned wounds, gingivitis, ear and larynx infections, lip and genital herpes, cystitis, chronic fatigue syndrome, tonsillitis, flu and grippe, tendinitis, sinusitis, mastitis, pneumonia, cut wounds, bruises, and abscesses [7].

The freeze-dried E. purpurea flower ethanolic extract has good antioxidant and antimutagenic activity [8].

Based on In vitro investigations results, ethanolic extracts of *Echinacea purpurea L.* can be inserted into treatment-healing chemotherapy for colon cancer due to the inhibition of cancer cell growth. This effect is strengthened by the addition of an inhibitory active ingredient, the Chic acid [9].

In India, the root (radix) used as an antivenin in the folk medicine. In Italy the dried leafs hot water extract has taken orally for inflammations in Italy [10].

In the case of autumn planting, no significant yield is expected in the following year. Only the 30% of the plants develop a flowering stem. Under unheated foil tent during early March (early spring) sowing seedlings can be planted in May. The best results are achieved in the case of November seedling and early spring plantings. In this case the time of planting will be shifted to the middle

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of summer, so later sowing is preferable. The planting in May is outstanding in the next year in growth and yield quantity [11].

Echinacea seeds soaked in polyethylene glycol or expanded vericulitis germinate at a higher rate and at a higher rate at 20 ℃ than the untreated ones [12].

In Clinical research in 2015 reports that a proprietary combination of a concentrated *Echinacea* herb and root extract is effective, when used early in the treatment of influenza [13].

The WHO keeps clinical data, which have been substantiated the root can be used for respiratory, tramadol infections and healing cold, the herba for skin diseases, and wounds. In the traditional American folk medicine it was used for fungal infections, radiation treatments and food poisoning [14].

Echinacea purpurea L. cough suppressant effect is similar to codeine. It' s bronchoconstrictive effect is more significant than other antiasthmatics used in clinical practice [15].

This plant is one of the most effective immune system amplifier feed supplement for horses [16].

Under an investigation in 2008, in the herba's active ingredients does not made significant change with the use of different nitrogen forms [17].

The *Echinacea purpurea* has a proved positive reaction for the organic fertilizers and the fertilizer dosing with the mass of the green herba, but the percentage distribution of the different plant parts does not significantly affected [18].

The highest amount of organic fertilizer applied had the highest achieved dry herb production, but the highest measured caffeic acid content was not in this setting [19].

In a nutritional supplement research they found that, neither the *Echinacea pallida*'s, nor the *Echinacea purpurea*'s *biomass production*, nor their selected active ingrediens̈t chosen as a marker did not show significant difference between the difference NPK nutrient supplies [20].

Not only the extraction solvent, but also the extraction conditions and the mode of the preparation are influencing the final bioactivity of herbal products. At the same time, the plant material itself plays a decisive role in this bioactivity too. According to test results, the aqua-alcoholic extract of the fresh plant has the most antioxidant effect [21].

II. Material and Methods

Our experiment took place in the experiment site of the University of Debrecen, Institute of Crop Sciences. Plot size was 8 m^2 and plots were arranged in 4 replicates in randomized blocks, with 6 different fertilizer treatment levels. The experimental place's soil is chernozem. The forecrops were potato and sunflower. In 2014 the regular annual nutrient dosages were spread on before we planned the experiment.

The fertilizer dosages of the experiment were spread manually in 2015 27th, 31th March, and 13th April, in 2016 29th March and in 2017 21th March. The intermittent fertilizer spread in 2015 was caused by the inappropriate weather conditions.

Sowing was 30th March in 2015 into seedling trays. The first plants were emerged 7th April. The planting were between 18th and 21st May. We planted the seedlings by hand in every plot, in 4 rows with 40 cm row space and 20 cm space between plants.

The first harvest of the flowering herba was 4th July in 2016. The second harvest was 10th July in 2017.

The fertilizer doses were:

- N0P0K0 (Control)
- N15P20K30
- N30P40K60
- N45P60K90
- N60P80K120
- N75P100K150

N%, P_2O_5%, K_2O%

The year 2015 was extremely dry. In this year the rainfall on the experimental area from 1st January to 30th September was considerably less (286.2 mm) than the 30-year average (445.8 mm). From January till the end of September the average temperature of each month were higher than the 30-year average (except the month of April).

In contrary to 2015, in 2016 the rainfall from 1st January to 31th August was considerably more (574.9 mm) than the 30 year average. From the 1st January to 31th August in 2016, the measured monthly mean temperature was higher than the 30 year average.

In 2017 it was remained below the 30 year average. In May when it was more than 30 millimeters "missing" compared to the average precipitation. The monthly measured mean temperature exceeded the 30 year average.

We measured the raw and the dry mass of the flowering herba, which we dried under prenumbra for three weeks in 2016. In 2017 because of the harvest time's rainy weather, we must use drying cabinet on 40 ^oC for 72 hours. During processing of the gained data, variance analysis, Pearson's correlation test and Factor analysis were applied by using MS Excel 2010 and IBM SPSS 22.0 programmes.

III. Results and Discussion

Fig. 1 shows the quantity and the changes of the raw herba yield of the coneflower depending on the nutrient supply in 2016 and 2017. In 2016 the control setting exceeded all nutrient settings' results. The mass of the measured herba decreased continuously, and reached the minimum in the N60P80K120 fertilization setting. In contrary to 2016, in 2017 the mass of the herba increased from the least nutrient dose, and reached the maximum in the N75P100K150 fertilization setting, and the control reached the lowest data.

We made single-factor variance analysis to investigate the connection between the quantity of the raw herba yield and the different nutrient settings. We did not find significant differences between the currently available data of the plots of different nutrient supply levels which is why the great standard deviation between the repetitions, however, we believe there is a relationship.

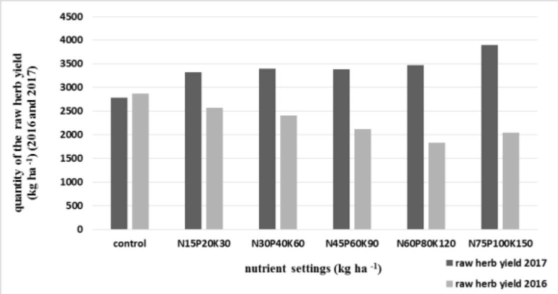

Figure 1. Quantity of the raw herb yield depending on the nutrient supply in 2016 and 2017 (Debrecen, 2017)

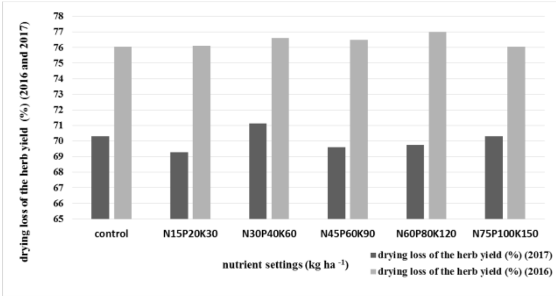

Figure 2. Drying loss of the herb yield of purple coneflower depending on the nutrient supply in 2016 and 2017 (Debrecen, 2017)

Fig. 2 demonstrates the drying loss of the coneflower's herba produced with the different nutrient settings in the two investigated year. In 2016 the drying loss was hectically increased and reached the maximum in the N60P80K120 nutrient requirement, than it was decreased. The highest value of the loss occurred in the N60P80K120 plots, followed by the N30P40K60, and the N45P60K90. The lowest drying loss was detected in the nutrient level N75P100K150, overtaken the control group. In 2017 the drying loss data were fluctuating. The highest value was measured in the N30P40K60 plots, followed by the control, and the N75P100K150. The lowest drying loss was detected in the nutrient level N15P20K30.

The difference between the two years' drying loss data could be explicable with the difference of the two years' rainfall conditions.

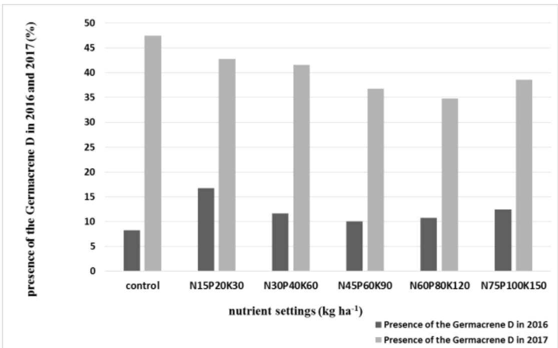

Figure 3. Presence of the Germacrene D in the herb of purple coneflower depending on the nutrient supply in 2016 and 2017 (Debrecen, 2017)

Fig. 3 shows the change of the presence of the Germacrene D in the coneflower's herb in 2016 and 2017. In 2016 we measured the highest presence in the N15P20K30, in 2017 the control settings. After these the presence starts to decrease. In 2017 the presence of the Germacrene D was multiplied by 2016.

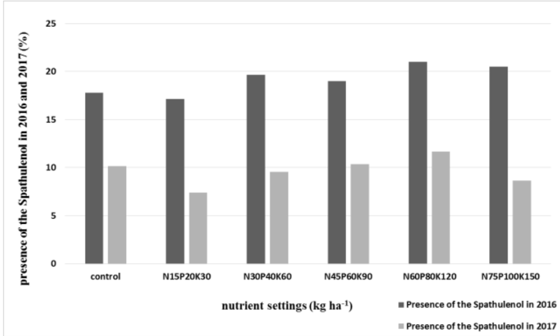

Figure 4. Presence of the Spathulenol in the herb of purple coneflower depending on the nutrient supply in 2016 and 2017 (Debrecen, 2017)

Fig. 4 shows the change of the presence of the Spathulenol in the coneflower's herb in 2016 and 2017. We measured the highest presence in the N60P80K120 nutrient settings in both years. From the lowest nutrient setting the presence was increased until the N75P100K150 fertilization settings. In 2016 the presence of the Spathulenol was multiplied by 2017.

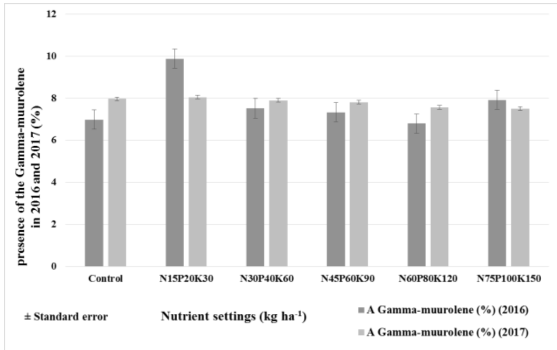

Figure 5. Presence of the Gamma-muurolene in the herb of purple coneflower depending on the nutrient supply in 2016 and 2017 (Debrecen, 2017)

In the case of the Gamma-muurolene – as it well observed on Fig. 5 - in the N15P20K30 nutrient setting's herb we measured the biggest presence in 2016 and 2017. After this setting, with the increasing fertilization the presence of the Gamma-muurolene decreased.

TABLE I. PEARSON CORRELATION TEST RESULTS OF THE PURPLE CONEFLOWER'S HERB ESSENTIAL OIL ACTIVE AGENTS (DEBRECEN, 2016-2017)

	Treatments	**Germacrene D**	**Gamma - m.**	**Spath.**
Treatments	1	-0,120	-0,222	-0,016
Germacrene D	-0,120	1	0,680**	0,558**
Gamma- m.	-0,222	0,680**	1	0,695**
Spath.	-0,016	0,558**	0,695**	1

*** significant P=0,01, * significant P=0,05*

Meanings:

- Gamma – m.: Gamma-muurolene
- Spath.: Spathulanol

We made a Pearson's correlation test to investigate the relationship between the essential oil active agents and the nutrient settings. Table I shows these relationships. The fertilization has not got significant effect for the essential oil active agent's presence. In contrast to this, we discovered a complex and complicated connection system between the different active agents. The Germacrene D has got a positive, significant (P= 0.01) relationship with the Gamma-muurolene (r= 0.680) and the Spathulenol (r= 0.558). The Gamma-muurolene has got a positive significant (P=0.01) relationship with the Sapthulenol (r= 0.695).

We made a Factor analysis, used the coneflower's herb, the essential oil active agent's and the eight weeks preharvest meteorological data (temperature, precipitation, humidity, soil temperature and global radiations). Based on the results of the analysis, in the investigation the meteorological conditions, and the vintage has got the biggest effect on the coneflower's drug yield and explains 85.59% of the total variance. These followed by the essential oil active agents (4,15%), then the nutrient requirements (2,83%) and the repetitions (2,6%).

IV. Conclusions

As for the raw herb drug yield, each nutrient setting was underlined the control setting in 2016, but in 2017, the N75P100K150 nutrient setting has the biggest, and the control group has the least measured yield.

In terms of the drying loss in 2016, the lowest value was reached by the N75P100K150 and the highest was produced by the N60P80K120 treatment. In 2017 we measured the highest loss in the N30P40K60, and the lowest in the N15P20K30 settings. The drying loss of the year 2016 and 2017 were fluctuating, which could be explicable with the difference of the two years' rainfall conditions.

As for the presence of the essential oil active agents for the Germacrene D in 2016 the N15P20K30 settings created the highest data, in 2017 the control group. In the case of the Spathulenol the N60P80K120 nutrient dosages brought the highest results. We measured the biggest presence in 2016 and 2017 for the Gamma-muurolene in the N15P20K30 nutrient settings. Based on our data, we think, there is not a strong relationship between the essential oil active agent's and the different nutrient dosages.

The made single-factor variance analysis to investigate the connection between the quantity of the raw flowering herba yield and the different nutrient settings did not show significant differences between the plots with the different fertilizer treatments. We did not find significant differences between the currently available data of the plots of different nutrient supply levels which is why the great standard deviation between the repetitions.

In our opinion the different weather conditions of the two examined years could have led to the conflicting results in the herb yield's, the drying loss and the active agent's data.

We made single-factor variance analysis with the data of the essential oil active agents which did not show any significant differences between the nutrient requirements too. The used Pearson's correlation test between the active agents and the fertilization settings. The results supported our idea that treatments have minimal impact on drug production. At the same time we discovered a connection network, a linkage between the essential oil agents. The correlations between these agents in several cases were medium strong at 1% significance level.

Based on the results of the Factor analysis the meteorological conditions, and the vintage has got the biggest effect on the coneflower's drug yield.

For the sake of clarity, we need more research work to clear up the complex connections between quantity of the coneflower's herb yield, it's essential oil agents and the effect of the different nutrient settings and the weather factors.

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