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Analysis of different fertilization settings' effect in the case of the summer savory's (*Satureja hortensis* L.) yield and active agents

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SUMMARY

During our research we investigated the Mediterranean summer savory's (Satureja hortensis L.) nutrient requirement with different fertilization settings in a small plot trial. We measured the medicinal plant's raw and dry drug mass, and the presence of the main essential oil active agents under the different fertilization settings using SPME (Solid phase microextraction) and GC-MS (Gas Chromatograph mass spectrometer).

From the drug's raw and dry mass perspective, based on the obtained results, in our opinion, the $N_{30}P_{40}K_{60}$ *nutrient requirements were ideal in 2015, while the* $N_{60}P_{80}K_{120}$ *nutrient requirement showed the best values in 2016.*

A significant relationship is assumed between the different fertilization settings and the essential oil active agents' distribution. During the analysis of data, we compared three of the savory's essential oil active agents' percentage of presence in the nutrient requirement samples.

Keywords: *herb, nutrient requirement, summer savory, essential oil*

INTRODUCTION

The growing of herbs is a very interesting boundary research area between arable and horticultural production. It is a special sector which needs more attention. Its producers and crops are trying to conquer more and more space in our country's agriculture. The herbs are the largest and most varied group among the cultivated plants with regard to the number of species. Similar diversity is shown only by the group of honey and ornamental plants (Rácz 1992). The herb's sector needs development in cultivation technologies and breeding, so that growers will be able to produce appropriate herbal drug substances at both the quality and the volume levels. The used herb species' culture level is still low nowadays. Hungary has few domestic breed varieties. In the best cases, we use cultivated populations, often with incomplete agrotechnical knowledge (Füleky 1999). In order for Hungarian herb farmers to succeed against foreign competitors, they need modern bred herb varieties and modern agrotechnical methods (Zámboriné 2010).

During the cultivation of medicinal plants, nutrient replenishment is significantly influenced by the drug production, which does not always mean the same part as in the case of conventional arable crops. Which part of the plant has the most active ingredients is different for each species? At the same time, the amount of active ingredients and their distribution are also affected by the applied nutrient.

In order to develop modern herbal cultivation, it is necessary to develop species-specific nutrient supplement to ensure economical yields while at the same time fulfilling European Union guidelines, quality assurance and environmental protection (Zámboriné et al. 2010). The chemical composition and nutritional requirements of plants depend on the conditions of cultivation and the variety used (Terbe 2007). There are still many uncertainties in determining the specific nutritional needs of herbs.

For the production of good quality and good active ingredients-containing drugs, the amount of nutrients taken and the total nutritional content applied are within wide limits (Valkovszki 2011).

The summer savory (*Satureja hortensis* L.) has a Mediterranean origin. Its drugs are the herba (*Saturejae herba*) and the essential oil (*Aetheroleum saturejae*) (Bernáth 2000). The single Hungarian species named „Budakalászi" in the National List of Species. Savory is mostly used in the food industry as pepper replacement and dietary spice and creator of blood-lifting tea blends (Takácsné Hájos 2004). Its cream can be used to heal bruises and rash, but it can also be used as mothballs. Its essential oil is skin rejuvenating, anti-inflammatory, most commonly used in toothpastes, soaps and in various vermouths (Marosi 2012). In the traditional healing it is used for low blood pressure, distention, pharyngitis and tonsillitis (Makay 1994).

The flowering herba contains 1–2% essential oil. The most specific essential oil ingredient is the p-cimol (20–30%) and the carvacrol (30–40%). In addition to the Mediterranean countries, there are many other *Satureja* species in the world, with different essential oil content of the active ingredient and their percentage. The Turkish *S. cuneifolia* also contains timol at some ripening stages. In Spain, in the same species' subspecies (*Satureja cuneifolia* subsp. L.), researchers found 35% camphor and 38% p-cimen. The *S. viminea* native in Jamaica, Cuba, Hispanola and Costa Rica contain 40% p-ment-3-en-8-ol and 35.3% pulegon. The different *Satureja species'* herba, its decoction and essential oil used in the other countries, just like in Hungary for appetizing spice, mild analgesic, antispasmodic, in the traditional healing (Héthelyi et al. 2002).

During the *S. rechingeri*, native in Iran, essential oil active ingredients' research it has been discovered that carvacrol, which is the main characteristic of one species group, has a negative correlation with several other active substances (Hadian et al. 2014).

Large amounts of terpenoid active ingredients in the *Satureja* species essential oil have antifungal effect, which was tested with the Iranian *S. knuzistanica*, the *S. spicigera* and the *S. hortensis* essential oil active ingredients effect *In vitro* conditions for strawberry fungus diseases (*Penicillium digitatum*, a *Botrytis cinerea* and the *Rhizopus stolonifer*) (Farzaneh et al. 2015). Terpenoids are secondary metabolic products, most commonly found in plants in nature.

The essential oil of the *Saturejae herba* contains monoterpens. The monoterpens have a characteristic odor, they are colorless, or pale yellow or green colour, and completely vaporized in free air. Their quality is affected both by soil, climate, the crop's age and the drug's collection date (Banai 2005).

Carvacrol and beta-bisabolene have been identified as potent antioxidants in the drug of *S. hortensis* (Samadi et al. 2015).

MATERIALS AND METHODS

Our small plot 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 replications in randomized blocks, with 6 different fertilizer treatment levels, in 4 rows with 40 cm row space. In 2015 on 2nd April and in 2016 on 4th April, sowing took place on the spot at 1 cm depth. Nutrients were applied manually. The first plants appeared on 20th April in 2015, and on 17th April in 2016. The first flowers were observed on 27th July in 2015, and on 4th July in 2016. The following fertilizer doses were applied:

- N 75 kg ha^{-1}, P_2O_5 100 kg ha^{-1}, K_2O 150 kg ha^{-1}
- N 60 kg ha^{-1}, P_2O_5 80 kg ha^{-1}, K_2O 120 kg ha^{-1}
- N 45 kg ha^{-1}, P_2O_5 60 kg ha^{-1}, K_2O 90 kg ha^{-1}
- N 30 kg ha^{-1}, P_2O_5 40 kg ha^{-1}, K_2O 60 kg ha^{-1}
- N 15 kg ha^{-1}, P_2O_5 20 kg ha^{-1}, K_2O 30 kg ha^{-1}
- The control plots did not receive nutrient supply.

The experimental place's soil is chernozem. In the previous year, before our research could be planned, the regular annual nutrient dosages were spread on the land which necessarily affected the yield. In 2014, the regular annual nutrient dosages were spread on the land. First on 5th March in 2014, 48 kg ha^{-1} nitrogen, 66 kg ha^{-1} phosphorus (P_2O_5) and 88 kg ha^{-1} potassium (K_2O) were applied. For the second time in the same year on 28th October, 38 kg ha^{-1} nitrogen, 31 kg ha^{-1} phosphorus (P_2O_5), and 37 kg ha^{-1} potassium (K_2O) were applied.

The rainfall on the experimental area in 2015 from 1st January to 30th September was considerably less (286.2 mm) than the 30-year average (445.8 mm). From January until the end of September, the average temperature of each month was higher than the 30-year average**,** except in April. 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.

The savory's flowering herba harvesting was done manually on 12th and 17th August in 2015 and from 8th to 10th August in 2016. The savories drying in 2015 first happened under prenumbra, and it took an average of three weeks. Because of the rainy weather of the first weekend of September the drug became wet again. Post drying was performed in a drying cabinet at 40 °C for 12 hours. In 2016, because of the rainy weather we dried the savory yield in a drying cabinet at 40 °C for 17 hours. We measured the drug yield in raw, dry and crumbled form in each plot. We stored the crumbled drug in paper bags. We also measured the loss of production of crumbed drugs. This was calculated using the data of the freshly harvested flowering herb and the weight of the crumbled dry herb by extracting 100% of the wet yield from the dry crumbled percentage relative to the former. The summer savory herba drug's quantity was decreasing during primary processing (drying and granulation). During this processing the drug's market value is increasing.

The analysis of the dried crumbled herb drug's essential oil components was carried out by applying SPME (solid phase micro extraction), then GC-MS (gas chromatograph-mass spectrometer). We used HP (Hewlett-Packard) 5890 Series II type gas chromatograph and 5971A type mass spectrometer. The detected essential oil components were identified by applying mass spectrums and Nist98 and Wiley databases. Active agents of the samples taken from each plot were analyzed. During the processing of the gained data, variance analysis and Pearson's correlation analysis were applied by using MS Excel 2010 and IBM SPSS 22.0 programs.

RESULTS AND DISCUSSION

In *Figure 1,* it can be observed that the savory raw drug yield changes as a result of the nutrient supply in 2015. Considering the quantity of the drug crop, the plots with $N_{30}P_{40}K_{60}$ had the most favourable nutrient setting, followed by the results of the plots with $N_{60}P_{80}K_{120}$, then that of the $N_{15}P_{20}K_{30}$ groups. The highest nutrient level ($N_{75}P_{100}K_{150}$) had the weakest effect on the quantity of the raw drug.

Figure 2 shows the crumbled drug yield of savory depending on the nutrient supply in 2016. In contrast with the raw yield data of 2015, the plots with $N_{60}P_{80}K_{120}$ had the most favourable nutrient setting, followed by the results of the plots with $N_{15}P_{20}K_{30}$, then those of the $N_{30}P_{40}K_{60}$ groups.

The three nutrient settings which provided the best results in 2015 and 2016 were $N_{15}P_{20}K_{30}$, $N_{30}P_{40}K_{60}$, and $N_{60}P_{80}K_{120}$.

The variance analysis did not show any significant differences between the different nutrient settings in wet conditions. One of the reasons for this may be the big deviation of crop data.

Figure 1: **The savory's raw drug yield in 2015 depending on the nutrient supply**

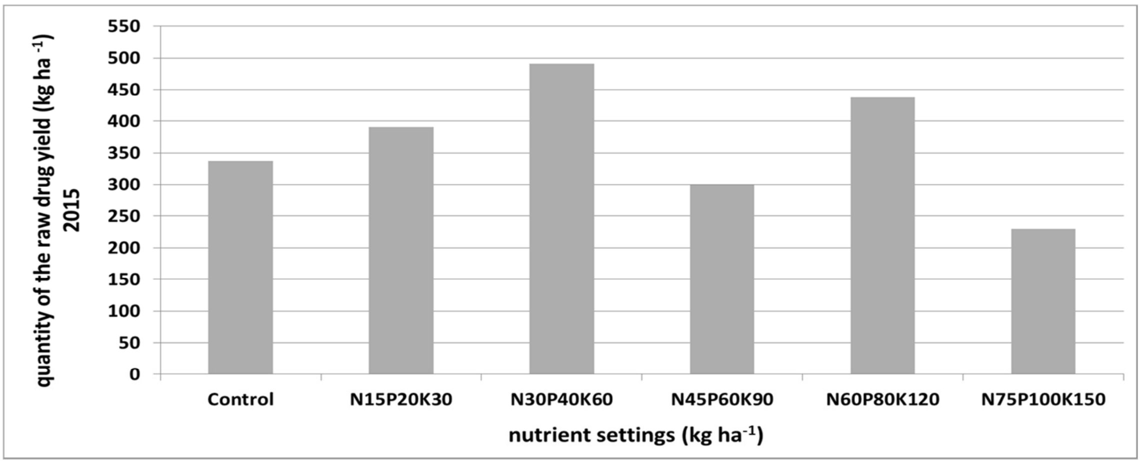
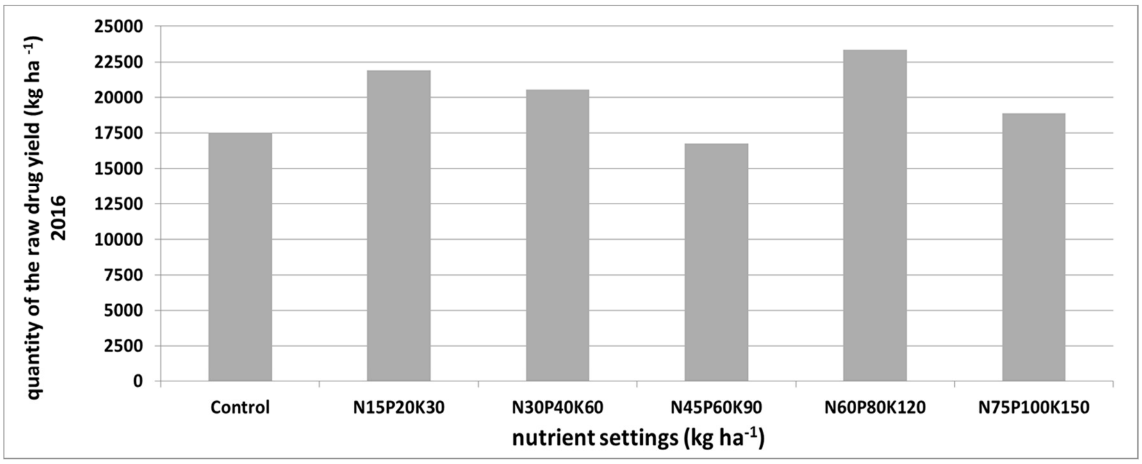
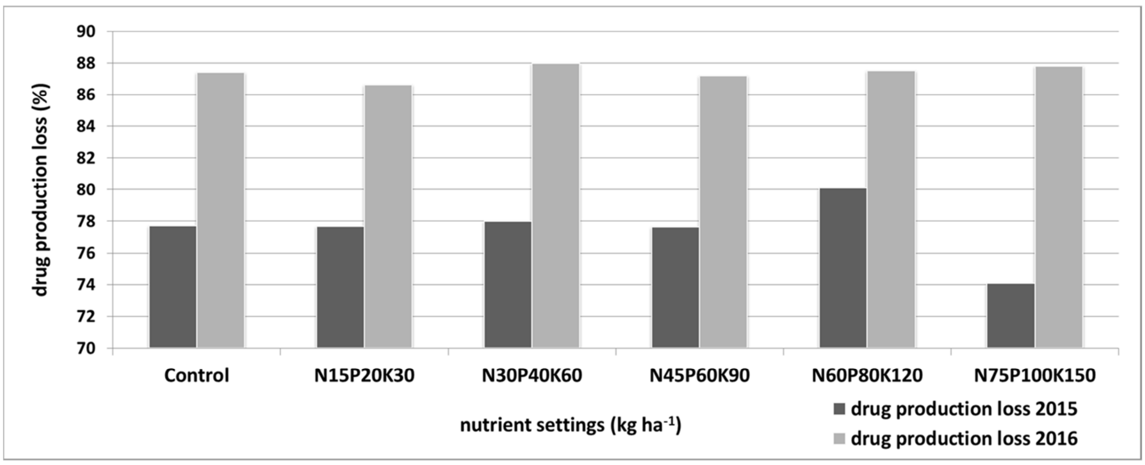


Figure 2: **The savory's raw drug yield in 2016 depending on the nutrient supply**



In 2015, the $N_{60}P_{80}K_{120}$ setting was the highest in terms of drug production loss (80.11%). The $N_{75}P_{100}K_{150}$ nutrient setting has the lowest loss data (74.07%). In 2016, thanks to the weather which was richer in rainfall, the summer savory drug production losses of the different nutrient settings grew higher than 86%. The highest value was measured in the $N_{30}P_{40}K_{60}$ setting, followed by the $N_{75}P_{100}K_{150}$ and $N_{60}P_{80}K_{120}$ settings (*Figure 3*).

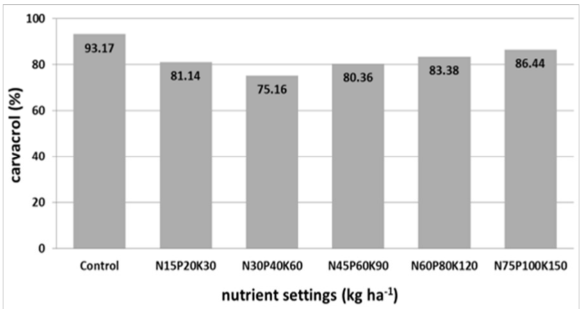
Figure 3: **The savory's drug production loss in 2015 and 2016 depending on the nutrient supply**



Carvacrol is one of the most characteristic components of essential oil of the summer savory. As a result of the treatments, its presence gradually decreased, until the $N_{45}P_{60}K_{90}$ set up again to increase, but all the way stayed down to the value of the control group.

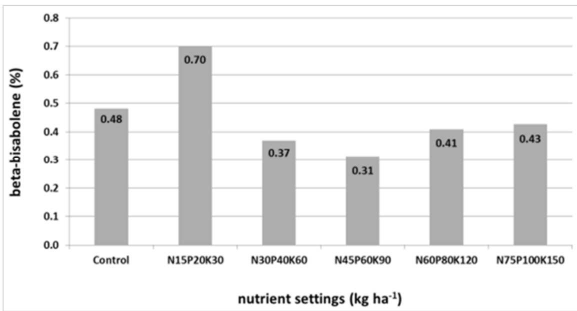
According to the results of the Pearson correlation test, the presence of this active ingredient in the essential oil of the summer savory and the different nutrient settings relationship is close to negative (r=-0.84) at 5% significance level. The examined cis-beta-terpineol (r=0.82) and beta-bisabolene (r=0.87) were in a significant relationship with a close 5% level (*Figure 4*).

Figure 4: **The carvacrol's presence in the savory's durg yield depending on the nutrition (2015)**



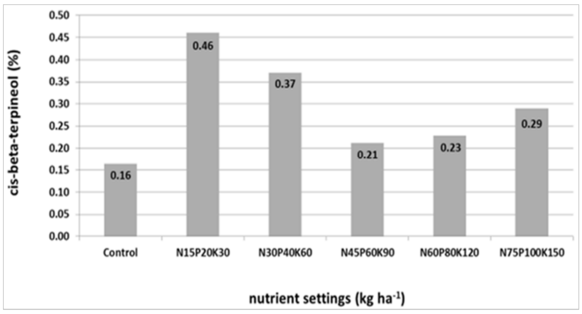
In *Figure 5*, it can be observed that the presence of the beta-bisabolene in the drug of savory depends on the nutrient supply. Outstanding quantity was measured in the $N_{15}P_{20}K_{30}$ fertilizer treatment. We detected a strong negative correlation (r=-0.64) between the presence of the beta-bisabolene in percentage and the increase of nutrient supply. There is a very strong correlation (r=0.87) between the presence of the beta-bisabolene and the carvacrol in percentage, and a strong correlation with the cis-beta terpineol (r=0.87) with at a 5% level of significance.

Figure 5: **The beta-bisabolene's presence in the savory's durg yield depending on the nutrition (2015)**



The largest amount of cis-beta-terpineol, similar to beta-bisabolene, was also measured in the $N_{15}P_{20}K_{30}$ nutrient setting (*Figure 6*).

Figure 6: **The cis-beta terpineol's presence in the savory's durg yield depending on the nutrition (2015)**



The cis-beta-terpineol active ingredient has a non-significant medium negative relationship with the increasing nutrient settings (r=-0.41). In contrary to this, with the carvacrol (r=0.82) and the beta-bisabolene (r=0.87) has a strong medium relationship on 5% significant level.

CONCLUSIONS

As for the raw drug yield, in 2015 the $N_{30}P_{40}K_{60}$ and the $N_{60}P_{80}K_{120}$ level was the ideal nutrient setting. In 2016, presumably because of the more rainfall, the $N_{60}P_{80}K_{120}$ and the $N_{15}P_{20}K_{30}$ level have achieved remarkable results. This positive effect was not substantiated by the results of the variance analysis. In our opinion, one of the possible reasons for this finding was the extremely dry and warm weather in 2015 and the warm and wet weather in 2016

The lowest drug production loss was measured in 2015 in the biggest nutrient setting ($N_{75}P_{100}K_{150}$). In 2016 this nutrient setting ($N_{75}P_{100}K_{150}$) has the second biggest loss.

According to the Pearson's correlation test results, the examined savory essential oil active ingredients presence and the different nutrient supplies has a variable strength negative relationship, at the 5% level of significance in the case of the carvacrol. The relationship between the different essential oil components is strong positive at the 5% level of significance in each case.

According to the obtained data, in our opinion, the increasing nutrient settings have a negative influence on the essential oil active agents' presence in the summer savory drug.

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