The marigold (*Calendula officinalis* L.) drug essential oil agents change under different fertilization settings in small plot trial

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SUMMARY

*During our research we investigated the marigold's (*Calendula officinalis *L.) nutrient requirements with different fertilization setting in small-plot trial. We measured 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.*

Based on the results, it was concluded, the essential oil agents' percentage breakdowns significantly depending on the cropping technologies. Besides that it is possible, based on Pearson's correlation test the marigold essential oil agents relationship can also be a major factor.

Keywords: *herb, nutrient supply, marigold, essential oil, active agent*

ÖSSZEFOGLALÁS

*Kutatásunk során a körömvirág (*Calendula officinalis *L.) tápanyagigényét vizsgáltuk különböző tápanyag-utánpótlási beállításokkal kisparcellás kísérletben. SPME (Szilárd fázisú mikroextrakció) és GC-MS (gázkromatográf-tömegspektrométer) alkalmazásával a különböző tápanyag-beállítások hatásait vizsgáltuk a növény főbb illóolaj hatóanyagainak százalékos megoszlására.*

Az eredmények alapján megállapítható volt, hogy az illóolaj hatóanyagok százalékos megoszlásában a termesztéstechnológia jelentős befolyással bír. Ezen kívül a Pearson-féle korrelációs vizsgálat alapján feltételezhető, hogy a körömvirág illóolaj-hatóanyagok kapcsolata egymással szintén jelentős befolyásoló tényező lehet.

Kulcsszavak: *gyógynövény, tápanyagellátás, körömvirág, illóolaj, hatóanyag*

INTRODUCTION

Phytotherapy is getting more and more emphasis in medicine (Nagy, 1994). The use and the cultivation of herbs in the XXI. Century is a re-discovered research field. There is an increasing need to develop modern, species and variety specific methods of nutrient supply that ensure profitable yields. In the same time we must comply with the directives of the European Union in terms of the requirements of quality assurance and environmental protection as well (Zámboriné et al., 2010). There are many uncertainties that give reason to doubt how to determine the specific nutrient requirements of herbs (Valkovszki, 2011). The statement that herbs are undemanding is incorrect (Zámboriné, 2010).

Marigold (*Calendula officinalis* L.) is West Asian origin, mediterranean medicinal plant (Rápóti and Romváry, 1987). As a drug, its flower is gathered with or without the speal (*Calendulae flos cum calycibus* and *Calendulae flos sine calycibus*) (Dános, 2006). The essential oil components content of marigold is 0.1%. One main agent is the Alpha-cadinol (Bernáth, 2000). It is slightly laxative and spasmolytic, but due to its high E vitamin content it is used for healing of the skin firstly. It is one of the most effective herbs that we can apply to treat lacerations, torn skin wounds and surgical scars and to alleviate itch occurring during wounds healing (Varró, 2011). A stable LGP (lamellar gel phase) emulsion is under development with using marigold, which can be an alternative to facilitate the healing of wounds (Okuma et al., 2015).

In our research we analyse the nutrient requirements and fertilizer reaction of marigold according to the change in the essential oil components and their distribution, as an effect of the different nutrient dosages.

The tested essetial oil active agents are terpenes include. Their common properties are the periodically recurring contracts and terminal methyl groups. The terpenes are volatile compounds, the major cause are the double bonds. Essential oils are non-uniform materials, their typical components are the terpenes. From the essential oil agents the terpenes can be monoterpenes and sesquiterpenes with opened or closed-chain. Their derivatives may also can be alcohols (Banai, 2005).

MATERIAL AND METHODS

Our experiment for the marigold research took place in the experiment site of the University of Debrecen, Institute of Crop Sciences. The experimental place's soil is chernozem. It is characterized by the accumulation of humus and easy tillage. There is not whitewash in the topsoil, it is prone to cracking in dry periods. The soil has got medium nutrient content, with good nutrient dynamics. The uniformly humus layer has a thickness of 50–70 cm. The water table is situated at depths of 7–9 m.

Plot size was 8 m^2 and plots were arranged in 4 replicates in randomized blocks, with 6 different fertilizer treatment levels, in 4 rows with 40 cm row space and 1 cm depth sowing. The sowing took place on the spot on 7th April 2015.

The fertilizer doses (N%, P_2O_5%, K_2O%) were come out like this:

- $N_0P_0K_0$ (control),
- $N_{15}P_{20}K_{30}$,
- $N_{30}P_{40}K_{60}$,
- $N_{45}P_{60}K_{90}$,
- $N_{60}P_{80}K_{120}$,
- $N_{75}P_{100}K_{150}$.

In the previous year, before our research could be planned, the regular annual nutrient dosages were spread on the land. First on 5 March 2014 48 kg ha^{-1} nitrogen, 66 kg ha^{-1} phosphorous (P_2O_5) and 88 kg ha^{-1} potassium (K_2O) were spread. The second nutrient supply took place on 28 October 2014 in the form of 38 kg ha^{-1} nitrogen, 31 kg ha^{-1} phosphorous (P_2O_5), and 37 kg ha^{-1} potassium (K_2O). The nutrient supply necessarily affected the yield. The fertilizer dosages of the experiment were spread manually.

The first emerged plants appeared on 20th April. The first flowers were observed on 5th June.

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 April).

Gathering was done 6 times manually between 6th July and 18th August 2015.

The analysis of the essential oil components was carried out in the NanoFood Laboratory by applying SPME (solid phase microextraction), then GC-MS (gas chromatograph-mass spectrometer). The sample preparation were manually. We used polyacrylate fibers with 85 µm size. The extraction time was one hour, on 50 °C temperature. The desorption temperature was 200 °C in the gas chromatograph injector. The time of desorption was 30 sec. We measured the samples for the SPME sampling in lockable pots with septum, which are qualifed for 20 ml airspace analysis. We made the extraction through the septum from the airspace.

During the GC-MS analysis the colonna were HP-5 stationary phase, 25 m×0.25 mm×0.25 µm capillary column. The carrier gas was helium with 1 ml min^{-1} flow rate, in 40 °C temperature, under constant injection pressure. The temperature of the analysis started from 50 °C two minutes, then the temperature was raised to 150 °C with 20 °C min^{-1} speed. After this we raised the temperature to 240 °C with 15 °C min^{-1} speed, which we held for 10 minutes.

The total analysis time was 23 minutes. The temperature of the injector was 200 °C, the injector liner was silanised liner without charge. The temperature of the transfer line was 280 °C. The ionization occurred on 70 eV, the mass range was 10–500 AMU (atomic mass unit). We used HP (Hewlett-Packard) 5890 Series II type gas chromatograph and 5971A type mass spectrometer. Components were identified by applying mass spectrums and Nist98 and Wiley databases. Active agents of the samples taken from each plot were analysed. During processing of the gained data, variance analysis and Pearson's correlation analysis were applied by using MS Excel 2010 and IBM SPSS 22.0 programmes.

RESULTS AND DISCUSSION

Figure 1 shows the values of Alpha-cadinol, one of the important essential oil components. The $N_{75}P_{100}K_{150}$ treatment was the most effective. According to the calculations, there is a medium correlation (r=0.52, P=5%) between presence of the Alpha-cadinol in percentage and the increase of nutrient supply.

Figure 1: **Presence of the Alpha-cadinol in the drug of marigold depending on the nutrient supply**

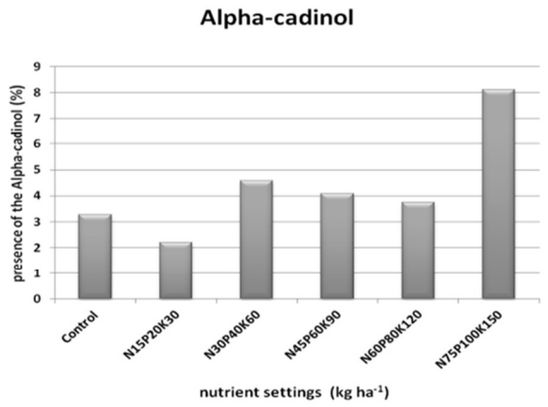


Figure 2 shows how the values of the Alpha-thujon changed with the different nutrient settings of the experiment. It is well demonstrated that after decreasing the $N_{15}P_{20}K_{30}$ treatment, presence of the active agent in percentage is continuously increasing. The highest value was measured with $N_{75}P_{100}K_{150}$ nutrient level, such as in case of the Alpha-cadinol.

Between presence of the Alpha-cadinol and the Alpha-thujon in percentage a very strong correlation (r=0.93, P=5%) was detected. Presence of the Alpha-thujon – as distinct from the Alpha-cadinol – has only a very weak correlation (r=0.25) with the nutrient settings.

Figure 2: **Presence of the Alpha-thujon in the drug of marigold depending on the nutrient supply**

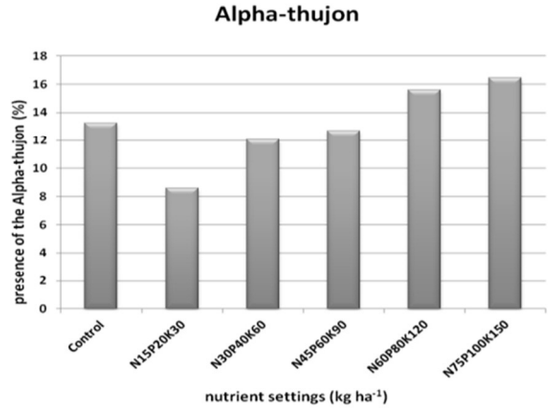
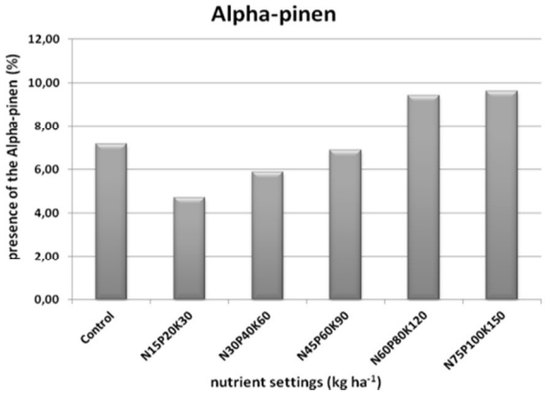


Figure 3 shows, how the values of Alpha-pinen changed with the different nutrient settings of the experiment. The $N_{15}P_{20}K_{30}$ fertilizer treatment reached the lowest measured values. The highest percentage value of presence was produced with the $N_{75}P_{100}K_{150}$

nutrient setting. The presence of the active agent in percentage from the $N_{30}P_{40}K_{60}$ is increasing.

Between the Alpha-pinen and the increasing nutrient dosages a very weak correlation (r=0.35) was detected. Between presence of the Alpha-cadinol and the Alpha-pinen in percentage a very strong correlation (r=0.94, P=1%) was detected.

Figure 3: **Presence of the Alpha-pinen in the drug of marigold depending on the nutrient supply**



It is clearly visible that on *Figure 4*, presence of the Alpha-cariophyllene active agent reached the highest value with the $N_{75}P_{100}K_{150}$ nutrient setting. The presence of the active agent is fluctuate from the control to the $N_{60}P_{80}K_{120}$ nutrient dosage.

Between the Alpha-cariophyllene and the increasing nutrient dosages a weak correlation (r=0.266) was observed. There was a very strong correlation (r=0.907, P=5%) between the presence of the Alpha-cariophyllene and the Alpha-cadinol in percentage in the dry drug of marigold.

Figure 4: **Presence of the Alpha-cariophyllene in the drug of marigold depending on the nutrient supply**

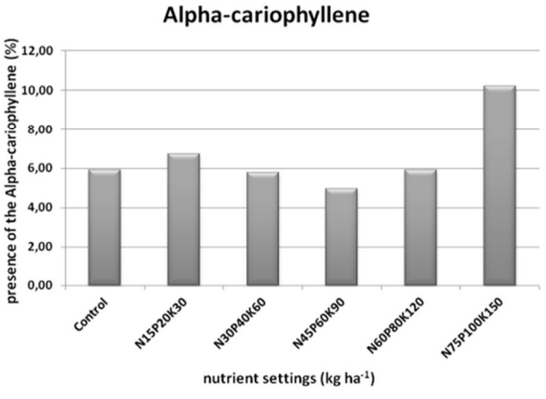
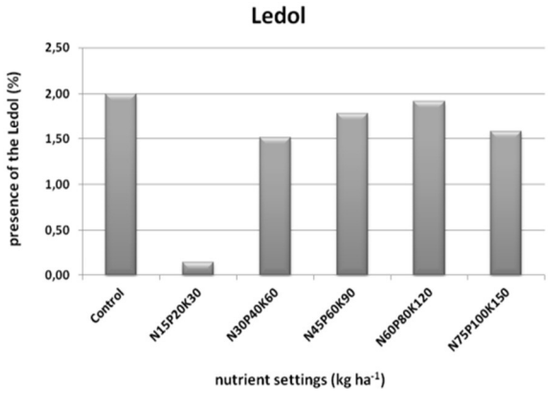


Figure 5 shows the values of the Ledol, which in itself is essentially a toxic compound. The Control group has the highest measured values. The lowest measured values was in the $N_{15}P_{20}K_{30}$ treatment. Then while the treatments increasing, the presence of the agent increasing also, but does not reach the level of the control group.

There is a weak correlation (r=0.33) between presence of the Ledol in percentage and the increase of nutrient supply. Between presence of the Alpha-cadinol and the Ledol in percentage a strong correlation (r=0.868) was detected.

Figure 5: **Presence of the Ledol in the drug of marigold depending on the nutrient supply**



It is clearly visible that presence of the T-cadinol reached the highest value with the $N_{75}P_{100}K_{150}$ nutrient setting. The presence of the active agent is fluctuate from the control to the $N_{60}P_{80}K_{120}$ nutrient dosage (*Figure 6*).

Between the T-cadinol and the increasing nutrient dosages a weak correlation (r=0.408) was measured. There was a very strong correlation (r=0.971, P=1%) between the presence of the Alpha-cadinol and the T-cadinol in the dry drug of marigold.

Figure 6: **Presence of the T-cadinol in the drug of marigold depending on the nutrient supply**

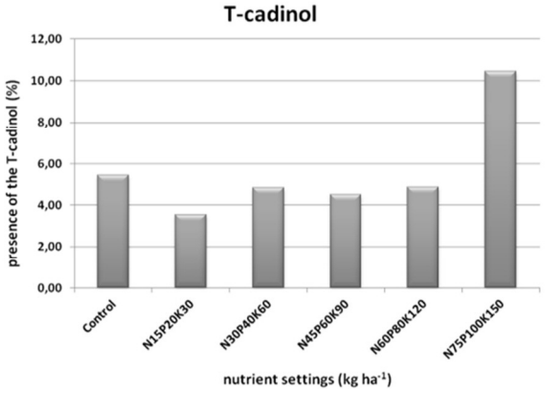
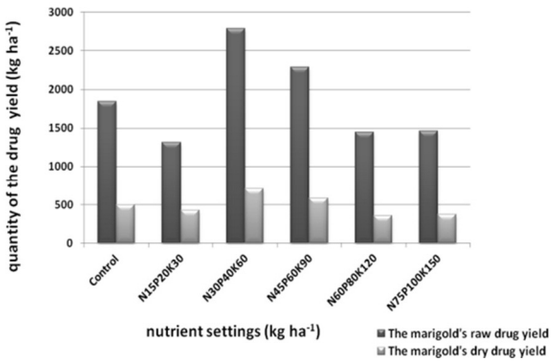


Figure 7 shows, the plots with $N_{30}P_{40}K_{60}$ had the most favourable nutrient setting, followed by the results of the plots with $N_{45}P_{60}K_{90}$, then that of the control groups, considering the quantity of the raw crop. The lowest nutrient level ($N_{15}P_{20}K_{30}$) had the weakest effect on the quantity of the raw drug. The mass data of the dry drug yield in proportions are similar to those of the raw drug. It can be observed too, the difference between the data of the raw and the dry yield is significant. The three nutrient setting – which provided the best results –

were similarly the $N_{30}P_{40}K_{60}$, the $N_{45}P_{60}K_{90}$ and the control setting. The dry drug yield produced with the nutrient setting $N_{15}P_{20}K_{30}$ was higher than that of the $N_{60}P_{80}K_{120}$ and $N_{75}P_{100}K_{150}$ was setting in contrary to the data of the raw crop.

Figure 7: **The marigold's raw and dry drug yield depending on the nutrient supply**



CONCLUSIONS

The nutrient supply has effect on the biomass production and yield of the crops also in case of herbs. Marigold's drug yield depended on the nutrient supply as well. Even so, we could not prove significant correlation between the presence of the essential oil active agents and the different nutrient settings based on the correlation analyses. We measured the highest percentage value of essential oil active agents with the $N_{75}P_{100}K_{150}$ nutrient setting except of Ledol.

Between the essential oil components in the dry drug there were strong and very strong correlations in P=5% and P=1% significance level. It is possible, based on Pearson's correlation test, that the relationship between the marigold's essential oil agents is the major factor. The connecting network of the active agents maybe has larger effect than the increasing nutrient doses. The fluctuation that occurred in the presence of the given active agents in percentage could also be caused by the different proportions of nutrients. We need more research work to do to clear the complex connections between the essential oil agents and the effect of the nutrient supply on the volatile oil content of the drug and the proportions.

As for the raw and the dry drug yield of the marigold, it seems the $N_{30}P_{40}K_{60}$ level was the ideal nutrient setting. In our opinion, one of the main reason for the fluctuation of the yield besides the different fertilizer dosages was the very warm and dry weather of the vegetative period. The variance analysis of the raw and dry drug mass' data did not show significant differences between the plots with different fertilizer treatments.

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