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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Abstract: While we investigated the marigold's (*Calendula officinalis L.*) nutrient requirements in small-plot trial we measured the raw and the dry drug yield, which we harvested in 2015 from 6th July to 17th August and in 2016 from 16 th June to 1 th August weekly. Thanks to the multiple picking the data we got, we investigated the effect of the different fertilization settings the flower drug's changes over time. The drug crop's change over time could show how many times it is economical to harvest under different fertilization settings. We monitored the harvested marigold's drug's raw and dry mass on a weekly basis. It was concluded, based on the results, in 2015 the biggest raw drug crop in the N30P40K60 fertilization setting and in 2016 in the N75P100K150 setting were measured. Both the raw and the dry mass' measurements of the case, this fertilization settings have the most important effect on the herb's yield. In the measurements of active ingredients we used SPME and GC-MS. We examined the effects of the different fertilization settings for the herb's main active ingredients of essential oil's percentage.

Keywords: herb, drug, nutrient supply, marigold, active agent

Inroduction

The *Calendula officinalis L.* (marigold, gold-bloom, Chinese safflower (WHO, 1999)) is a mediterranean annual plant (Rápóti and Romváry, 1987). The drug of this medicinal plant is the flower (Dános, 2006). The essential oil is the 0.1 % percent of the total active ingredient content. (Bernáth, 2000). Because of it's high E vitamin content mainly it is used for healing of the skin (Varró, 2011). For horses' the marigold could be used the follow-up care of fractures, bruises and sprains. Internally used to treat stomach ulcers (Marton, 2005).

Research is under the way for a stable LGP (lamellar gel phase) emulsion with using marigold, which could be an alternative to facilitate the healing of wounds (Okuma et al., 2015). Under diabetes, because of the high blood sugar the non-enzymatic glycation of the proteins speed up, and the end products are accumulate int he body. This promotes the typical complications of diabetes, and leading to arterial stiffness that decreases myocardial compliance and aging. The marigold had greater antioxidation potential, which could inhibit these two reactions.(Ahmad et. al, 2012) The diabetic patients limbs' micro traumas are constant possibility of infections, which could lead to amputation. The marigold balm stopped the formation and progression of infections reduced the itching, the redness, the dryness, and the pain. Contributed to the disappearance of scars, while the risk of allergy to the plant is very low (Cioinac, 2016). Under our research we analysed the nutrient requirements and fertilizer reactions of marigold according to the change in the drug yield and the essential oil components distribution, as an effect of the different nutrient dosages. It has been established – under investigations - the different fertilization settings has not got significant influence on the marigold's flavonoids, but the amount of light and the plants' age (Fernandes et al., 2013). The marigold's essential oils' typical agents – which we investigated - are the terpenes. They have got antifungal antibacterial and antiviral effects (Banai, 2005).

Materials and methods

Our experiment took place in the experiment site of the University of Debrecen, Institute of Crop Sciences. 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. The nutrient supply necessarily affected the yield. 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. In 2015 and 2016, sowing took place on the spot on 7th April and on 4 th April, in 1 cm depth. The fertilizer doses:

- N0P0K0 (Control)
- N15P20K30
- N30P40K60
- N45P60K90
- N60P80K120
- N75P100K150 N%, P_2O_5%, K_2O%

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 till the end of September the average temperature of each month were higher than the 30 year average**,** except 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. We measured the drug yield which, in this case, was the quantity of the raw and the dry drug. Gathering was done 6 times manually between 6th July and 18th August 2015 and 7 times in 2016 between 16 th June and 1 th August. Analysis of the essential oil components was carried out by applying solid phase microextraction (SPME), then gas chromatograph-mass spectrometer (GC-MS). 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.

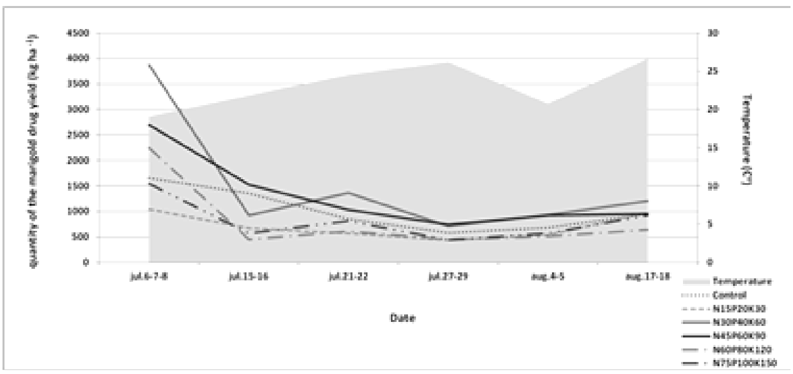

Figure 1.The connection between the marigold raw drug yield and the average temperature in 2015 (Debrecen, 2015)

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Results and discussion

Figure 1. shows the relationship between the marigold raw drug yield and the average temperature in 2015. Considering the quantity of the raw drug yield, the plots with N30P40K60 had the most favourable nutrient setting, followed by the results of the plots with N45P60K90, then that of the control groups. During the harvest all of the plots' yield decreased. Between 21th and 22th July and between 17th and 18th August we measured a weak growth which was the biggest in N30P40K60 fertilizer treatment. The temperature

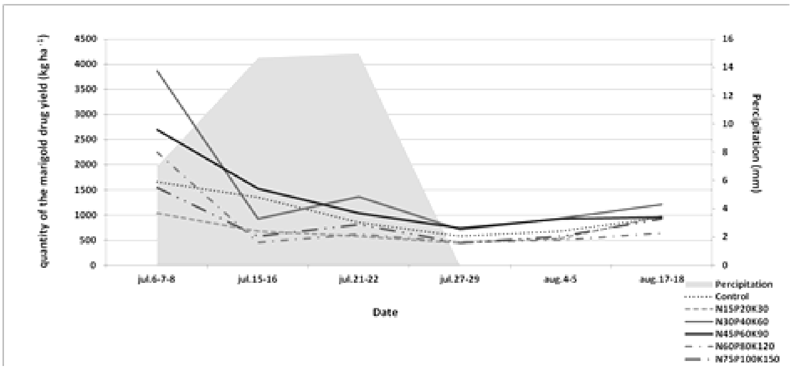

Figure 2.The connection between the marigold drug yield and the percipitation in 2015 (Debrecen, 2015)

except in 4th and 5th August grown.We investigated the relationship between the raw drug yield and the temperature with Pearson's correlation test. We measured that, there is a negative medium connection between them (r= - 0.34, P=1%).

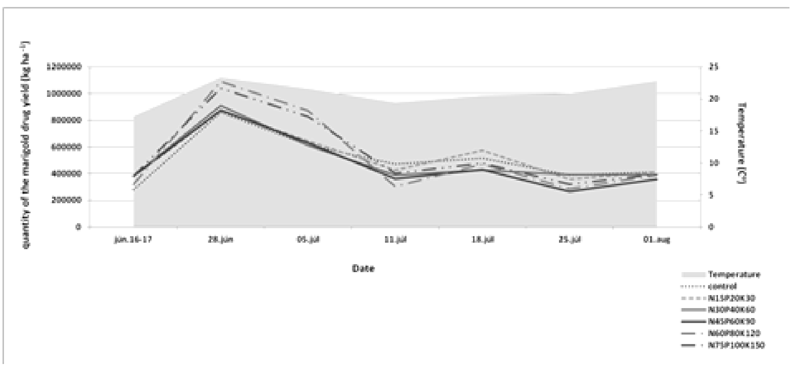

Figure 3.The connection between the marigold drug yield and the average temperature in 2016 (Debrecen, 2016)

Figure 2. shows the relationship between the marigold raw drug yield and the percipitation in 2015. From the first harvest (6th-7th-8th July) the percipitation approximately doubled. Then the rainfall start to decrease and scheduled for 27th July loss. We measured only a very weak not significant correlation between the the raw drug yield and the precipitation (r=0.106).

Figure 3. shows the relationship between the marigold raw drug yield and the average temperature in 2016. In 2016 the N75P100K150, the N60P80K120 and the 15P20K30 fertilization setting has the biggest effect to the raw drug yield, and the N45P60K90 setting has the weakest effect on

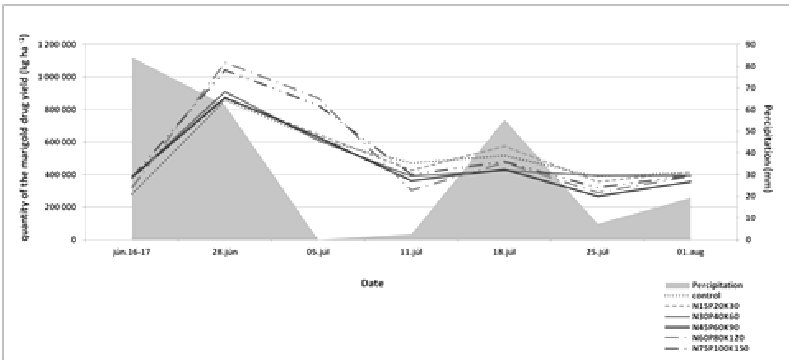

Figure 4.The connection between the marigold drug yield and the percipitation in 2016 (Debrecen, 2016)

the full raw yield. During the harvest the plots' yield firstly increased. We measured the highest yiel in the harvest of 28th June. Then the quantity of the yield start to decrease. There were a slight increase again on 18th July, but the yield reduction then continued. Until 11th July the N60P80K120 and the N75P100K150 settings' yields reduced least.We found a positive medium correlation (r=0.39, P=1%) between the raw drug yield and the temperature.

Figure 4. shows the relationship between the marigold raw drug yield and the percipitation in 2016. From 5th July to 11th July there was a minimum in rainfall. After this period, the percipitation increased until 18th July. The next minimum int he rainfall was in 25th July and then started a new increase. The Pearson's correlation test showed a very weak, not significant relationship between the raw drug yield and the percipitation (r=0.08).

During the joint analysis of the 2015 and 2016 years datas we measured a medium correlation (r=0.38, P=1%) between the raw drug yield and the percipitation. There were a negative weak relationship (r= - 0.18, P=1%) between the marigold raw yield and the average temperature.

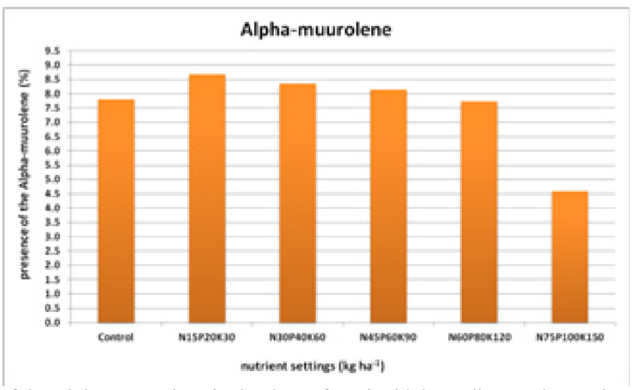

Figure 5 Presence of the Alpha- muurolene in the drug of marigold depending on the nutrient supply (Debrecen, 2015)

During the harvest, the increase of the number of the harvests, the yield was decrease – the experienced relationship between the number of the harvests and the drug yield is negative but not significant (r= - 0.10). We made a variance analysis between the data of the drug yield and the plots with different fertilizer treatments, but it did not show significant differences.

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We analysed the marigold drug's essential oil active agents percentage presence depending on the increases of the applied nutrient doses. These active agents, which we identified are mostly belong to the group of terpenes. We analysed the presence of the Alpha-muurolene and the Gamma-muurolene.

The highest presence of the Alpha-muurolene what we measured was in the N15P20K30 nutrient setting. It was reported a higher percentage from this agent in the N15P20K30,

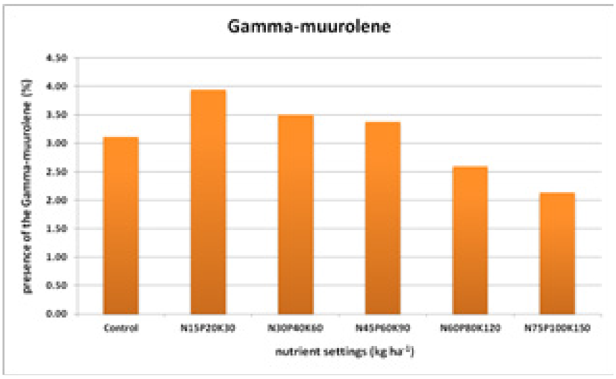

Figure 6 Presence of the Gamma- muurolene in the drug of marigold depending on the nutrient supply (Debrecen, 2015)

N30P40K60 and the N45P60K90 than in the Control group. Hereinafter the presence of the Alpha-muurolene was decreased with the increasing of the applied nutrient doses (*Figure 5.*). We also used the Pearson's correlation test to analyse the relationship between the presence of the essential oil active agents and the increasing nutrient settings. In the case of the Alpha-muurolene we found strong negative, but not significant correlation (r= - 0,79).

We also measured the highest presence of the Gamma-muurolene in the N15P20K30 nutrient supply. As well a sin the case of the Alpha-muurolene, in the N15P20K30, N30P40K60 and the N45P60K90 were reported higher percentage than in the Control group. Then the following groups have occurred the decrease of the Gamma-muurolene.
The correlation test showed a strong medium negative, not significant relationship between the presence of the Gamma-muurolene and the increasing of the nutrient settings (r= - 0,69).

Conclusions

Based on the correlation analysis in 2015 there were a negative relationship between the raw marigold drug yield and the temperature (r= - 0.34, P=1%), and a weak positive between the raw marigold drug yield and the percipitation (r=0.106). In 2016 there were a positive medium correlation between the raw marigold drug yield and the temperature (r=0.39, P=1%), and a very weak positive between the raw marigold drug yield and the percipitation (r=0.08). Analyzing the two years' datas together we find a negative medium correlation between the raw marigold drug yield and the temperature (r=0.38, P=1%), and a weak positive between the raw marigold drug yield and the percipitation (r= - 0.18, P=1%).

Because of these correlation results, and the variance analysis's not showed significance, in our opinion the changes of the temperature and the rainfall are more effective for the marigold's drug yield than the increasing nutrient settings. This phenomenon can influence the changes of the harvests' number, as well as the possible renewal term of the stand. Based on the results of the Pearson's correlation test between the marigold drug's essential oil active agents and the nutrient supplies, we think, the increasing nutrient supply has a negative effect on the presence of the essential oil active agents in the marigold's drug, but until we can not make more tests, we could not say with certainty, there is relationship between the presence of the essential oil active agents and the different nutrient settings.

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