RELATIONSHIP INVESTIGATION BETWEEN THE MARIGOLD (*CALENDULA OFFICINALIS* L.) ESSENTIAL OIL AGENTS AND QUANTITATIVE PRESENCES CHANGE UNDER DIFFERENT FERTILIZATION SETTINGS

Lelesz Judit Éva*, Csajbók József**

*PhD hallgató, Debreceni Egyetem, Növénytudományi Intézet,
e-mail: lelesz.judit@agr.unideb.hu
**Egyetemi docens, Debreceni Egyetem, Növénytudományi Intézet

Abstract

During our research we investigated the marigold's (Calendula officinalis L.) essential oil active agents quantitative presences change 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 of their presence in the drug.

Key words: herb, nutrient supply, marigold, essential oil, active agent

INTRODUCTION

In the XXIth century, the interest is growing after their production and using. The group of herbs contains most species compared to the other groups of crops.

Phytotherapy is getting more emphasis in traditional medicine (Nagy, 1994).

According to a survey, more than the half of the consumers are using medicinal pplants weekly. The most important factors under the shopping are the quality and the price and after these comes only the country of the origon. The most often mentioned consumer motivations are the own ideas, and the friend's reviews. The effect of the doctor's proposals and the media are minimal (Panyor, 2012).

The use and the cultivation of herbs in the XXIth Century is a re-discovered research field. The state of today's medicinal plant's market is a little chaotic. The export datas of the 90's were disappeared, except those hungaricum products which quality still in Hungary are the highest, thanks to the good ecological conditions (Hornok, 1986). In order to these products will be able to get us recognition on the world market, we need to renew the sector strategy on macro and micro level too, which's validation could be successful with an effective advisory system (Bernáth, 1992). To protect the traditional reputation we must act consciously, which includes the research and development the job training, and the development of the infrastructure (Zámboriné, Bernáth, 2003). To keep the Hungarian herbal sector's competitiveness, we need to make the basic conditions in appropriate strategy and support system (Bernáth, 2003).

There is an increasing need to develop modern, species and variety specific methods of nutrient supply that ensure profitable yields and the directives of the European Union about quality assurance and environmental protection must be complied too (Zámboriné et al., 2010). There are many uncertainties in the herbs specific nutrient requirements (Valkovszki, 2011).

The marigold (*Calendula officinalis L.*) has a West Asian origin. It is a Mediterranean medicinal plant (Rápóti, Romváry, 1987). As a drug, it's flower is gathered with or without the sepal (*Calendulae flos cum calycibus and Calendulae flos sine calycibus*) (Dános, 2006).

It is also known as chinese safflower, fleurs de tous les mois, garden marigold, gold-bloom, goldblume and pot marigold (WHO, 1999). This last name historically associated with it's use in soups and stews for combat illnesses (Ramos et al., 1988). Nowadays the marigold is approved for food use in U.S.A. and appears in the Food and Drug Administration's list of Generally Recognized as Safe (GRAS) substances (Della-Loggia et al., 1994). A number of reports observed in it's drug sesquiterpene glycosides, saponins, xanthophylls, triterpenes, and flavonoids. Because of the economic value as a medicinal plant and the general use in cosmetics, perfumery, pharmaceutical preparations, and food production, it is important to study the composition of essential oil in *Calendula officinalis L.* (Gazim et al., 2008).

The essential oil components content of marigold is 0.1% (Bernáth, 2000). It is slightly laxative and spasmolytic, but due to its high Vitamin E content it is used for healing the skin first. 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). It could be used to horses' fractures, bruises, sprains and tears follow-up care. Internally for cancerous tumors, stomach, intestines, and other colic convulsion and mainly used to treat stomach ulcers with chamomile brew (Marton, 2005). Szabó and Bujdosó observed that in their hospital ward for limbs and bedsores the used calendula more quickly removes dead and viable tissue sections. It is good to use for the bedsore's prevention and curing too (Szabó, Bujdosó, 1994). The shepherds to cure panaritium disease bathe the treated leg in the alcoholic extract of marigold (Draskóczy, 1996).

Under Fernandes et al. (2013) investigation has been established the different fertilization settings has not got significant influence on the Calendula officinalis' flavonoids. In the same time in the seasonality were significant differences. It is possible not the fertilization is the most effective, but the amount of light and the plants' age.

In our research we analysed 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 essential oil active agents we tested are include in the terpenes group. The marigold essential oils are non-uniform materials, their typical components are the terpenes. Above the essential oil agents the terpenes can be monoterpenes or sesquiterpenes with opened or closed-chain (Banai, 2005).

MATERIAL AND METHOD

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. 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.

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.

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 were:

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

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. 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. The total analysis time was 23 minutes. 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. 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

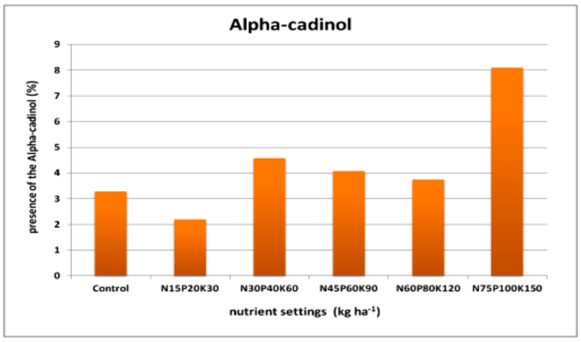

Fig. 1. Presence of the Alpha-cadinol in the drug of marigold depending on the nutrient supply (Debrecen, 2015)

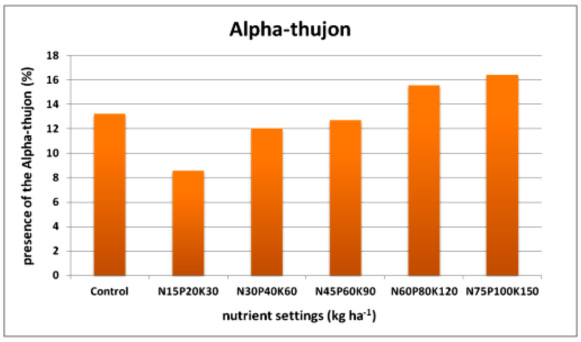

Fig. 2. Presence of the Alpha-thujon in the drug of marigold depending on the nutrient supply (Debrecen, 2015)

Figure 1 shows the values of Alpha-cadinol, one of the important essential oil components. The N75P100K150 treatment was the most effective. According to the calculations, there is a correlation (r=0.52, significant on 5% level) between presence of the Alpha-cadinol in percentage and the increase of nutrient supply. Between presence of the Alpha-cadinol and the Alpha-thujon in percentage a very strong correlation (r=0.93) was detected. Between the presence of the Alpha-cadinol and the T-cadinol we measured a very strong correlation (r=0.97, significant on 1% level).

Figure 2 shows how the values of the Alpha-thujon changed with the different nutrient settings of the experiment. After decreasing the N15P20K30 treatment, presence of the active agent in percentage is continuously increasing. The highest value was measured with N75P100K150 nutrient level, such as in case of the Alpha-cadinol. Presence of the Alpha-thujon does not have a strong correlation (r=0.25) with the nutrient settings. We measured correlation between the presence of the Alpha-thujon and the T-cadinol (r=0.95, significant on 1% level).

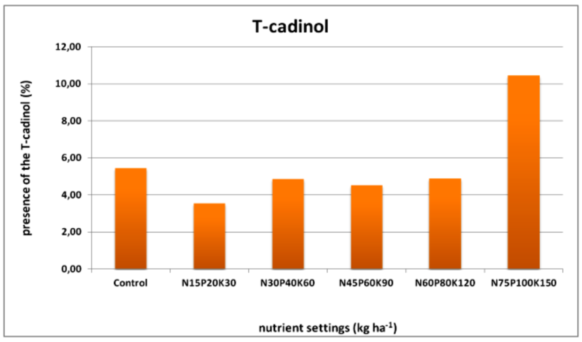

Fig. 3. Presence of the T-cadinol in the drug of marigold depending on the nutrient supply (Debrecen, 2015)

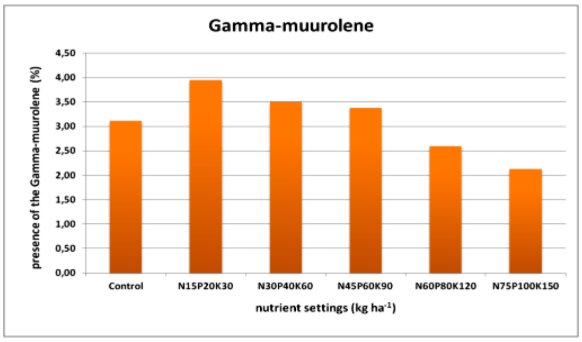

Fig. 4. Presence of the Gamma-muurolene in the drug of marigold depending on the nutrient supply (Debrecen, 2015)

On Figure 3 the presence of the T-cadinol reached the highest value with the N75P100K150 nutrient setting. It is clearly visible that presence of the active agent is fluctuating from the control to the N60P80K120 nutrient dosage.

Between the T-cadinol and the increasing nutrient dosages there is a weak correlation (r=0.408) was measured. There was a very strong correlation (r=0.971, significant on 1% level) between the presence of the Alpha-cadinol and the T-cadinol in the dry drug of marigold. Between the presence of the T-cadinol and the Alpha-thujon we measured a very strong correlation (r=0.95, significant on 1% level).

It is clearly visible on the Figure 4 that presence of the active agent reached the highest value with the N15P20K30 nutrient setting. Further increase of the nutrient supply resulted in coherent decrease of the active agent. We could not measured strong correlation between the Gamma-muurolene and the Alpha-cadinol (r=0.05), the Alpha-thujon (r=0.20), and the T-cadinol (r=0.07).

CONCLUSIONS

The nutrient supply has effect on the presence of the essential oil active agents in the Marigold's drug, but 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 N75P100K150 nutrient setting except of Gamma-muurolene.

Between the essential oil components in the dry drug there were not in every situation strong and very strong correlations in P=5% and P=1% significance level. Nonetheless we think it is possible, based on Pearson's correlation test, that the relationship between the marigold's essential oil agents is the major factor and has larger effect than the increasing nutrient doses.

We need more research work to do to clear the complex connections between the essential oil agents and the effect of the different nutrient settings.

REFERENCES

1. Banai V., 2005, Gyógynövény- és drogismeret. Műszaki Könyv Kiadó, 2. kiadás, Budapest, pp.258
2. Bernáth J., 1992, A gyógynövény szaktanácsadás és annak speciális ágazati kérdései. Kertészet, Szaktanácsadási szekció II. kötet, pp.608-611
3. Bernáth J. (Szerk.), 2000, Gyógy- és aromanövények. Mezőgazda Kiadó, Budapest
4. Bernáth J., 2003, A gyógynövény ágazat lehetőségei és korlátai az EU csatlakozás során. Az Európai Unió Agrárgazdasága, 8. évf., 3. sz. pp.17-20
5. Dános B., 2006, Farmakobotanika, Gyógynövényismeret, Harmadik, bővített, átdolgozott kiadás, Semmelweis Kiadó, Budapest

6. Della-Loggia R., Tubaro A., Sosa S., Becke R.H., Saar S.T., Isaac D., 1994, The role of triperpenoids in the topical antiinflamatory activity of Calendula officinalis flowers. Planta Med., v.60, pp.516-520
7. Draskóczy J., 1996, A panaritium kezelése körömvirág-kivonattal Magyar állatorvosok lapja. 51. évf., 6. sz., pp.380
8. Fernandes E.F.A., Meloni F., Borella J.C., Lopes N.P., 2013, Effect of fertilisation and harvest period on polar metabolites of Calendula oficcinalis. Revist Brasileira de Farmacognosia (Brazilian journal of pharmacognosy), 23. évf., pp.731-735
9. Gazim Z.C., Rezende C.M., Fraga S.R., Filho B.P.D., Nakamura C.V., Cortez D.A.G., 2008, Analysis of the essential oils from Calendula officinalis growing in Brazil using three different extraction procedures. Revista Brasileira de Ciências Farmacêuticas Brazilian Journal of Pharmaceutical Sciences, vol. 44, n. 3, jul./set.
10. Hornok L., 1986, A gyógynövénytermesztés eredményei és termelésfejlesztési feladatai. Kertgazdaság, 18. évf., 3. sz., pp.47-52
11. Marton Z.S., 2005, Lóherba, Gyógynövények lovaknak, Equinter Kiadó, Budapest
12. Nagy G., 1994, Gyógynövény-történelem, Recept: független egészségpolitikai magazin, 5. évf., 10. szám, pp.22-23
13. Okuma C.H., Andrade T.A., Caetano G.F., Finci L.I., Maciel N.R., Topan J.F., Cefali L.C., Polizello A.C., Carlo T., Rogerio A.P., Spadaro A.C., Isaac V.L., Frade M.A., Rocha-Filho P.A., 2015, Development of lamellar gel phase emulsion containing marigold oil (Calendula officinalis) as a potential modern wound dressing. European Journal of Pharmaceutical Sciences, 71 (2015), pp.62-72
14. Panyor Á., 2012, Gyógynövény-fogyasztási szokások. Marketing & menedzsment, 46. évf., pp.88-95
15. Ramos A., Edreira A., Vizoso A., Betancourt J., López M., Décalo M., 1988, Genotoxicity of an extract of Calendula officinalis L.. J. Ethnopharmacol., v.61, pp.49-55
16. Rápóti J., Romváry V., 1987, Gyógyító növények. Medicina Könyvkiadó, Budapest
17. Szabó E., Bujdosó J., 1994, Biotermék a gyógyászatban – körömvirág. Nővér, 7. évf., 3. sz. pp.20
18. Valkovszki N.J., 2011, Egyéves konyhakömény tápanyagigényének vizsgálata. Agrofórum, 22. évf., 3. szám, pp.102-104
19. Varró A.B., 2011, Gyógynövények gyógyhatásai, Növényi gyógyszerek, Hazai gyógynövényeink ismertetése, gyűjtésüknek módja és felhasználásuk a mindennapi életben az egészség szolgálatában, Kódexfestő Könyvkereskedés Kft., Debrecen, Kinizsi nyomda
20. Zámboriné N.É., Rajhárt P., Szabó K., És Antal T., 2010, A tápanyag-utánpótlás hatása gyógynövények hozamára és drogminőségére. Kertgazdaság, 42. évf., 3-4. szám, pp.128-135
21. Zámboriné N.É., Bernáth J., 2003, Gyógy-és aromanövény hungarikumok megőrzése és fejlesztése. Kertgazdaság, 35. évf., 1. sz., pp.105-110
22. ***, 1999, Monographs on selected medicinal plants. WHO, Vol. 1