ANTIOXIDANT POLYPHENOL AND FLAVONE CONTENTS IN CORRELATION WITH CULTIVATION TECHNOLOGY FOR *CALENDULA OFFICINALIS* L.

Georgeta POP*, Ersilia ALEXA*, Andreea Viorica MILITARU**

Banat's University of Agriculture Sciences and Veterinary Medicine Timisoara, Romania
**University of Medicine and Pharmacy. "Victor Babes Timisoara*
Corresponding author: Georgeta Pop, Banat's University of Agriculture Sciences and Veterinary Medicine Timisoara, 119 Calea Aradului, 300645 Timisoara, Romania, tel.: 0040256277057, fax: 0040256488070, e-mail: getapop_tm@yahoo.com

Abstract. During the last two decades there has been a strong trend to returning to plant therapy. Research highlight polyphenol and flavone content of *Calendula officinalis L* playing an important role in protecting man's health. Natural antioxidants are spread particularly in the plant kingdom[2]. Bio-flavones and polyphenols are man's main allies in his fight against all threats to body's health, including viruses, cancer,, toxic substances and micro-organisms. Antioxidant action of polyphenols is largely used in medicine and pharmacy as deactivating agents of ultraviolet radiation and metal ions. These substances decompose peroxides and inhibit the forming action of free radicals, thus hindering disease appearance [3]. The paper aims to establish that the yield capacity of the analysed cultivars was significant influenced by the cultivation technology. Sowing densities is one of the most important technological step for marigold. Fertilisation also had great influence on yield capacity. Concerning the polyphenol content and the flavones content we can observe that the technology had great impact on their quantity and quality, so in order to obtain best results we should take in consideration the fact that is vital to improve the cultivation technology for marigold. The quality of marigold yield is given by the content of active elements from the inflorescences. From these elements we study in particularly the polyphenols and the flavones.

Keywords: plant therapy, marigold, yield, technology, polyphenol content

INTRODUCTION

The World Health Organisation estimated that 75-80% of the rural population on the Globe appeals to traditional medicine. This resulted in an intensification of the efforts in research and development of traditional medicine activities, to use for man's benefit this real source of information concerning plant properties [10]. Medicinal and aromatic plants are herbaceous, annual, biannual, perennial, or woody species, from which they use the leaves, inflorescence, fruit, roots, etc. The most important pharmacodynamic use of flavones is as vitamin P. They play a role in blood vessel permeability, thus increasing capillary wall resistance and hindering haemorrhage. The role of flavonic derivatives in the process of cell oxido-reduction was highlighted [3]. Flavones also enhance the action of ascorbic acid, weakening the action of histamine by inactivation and protect the body against the noxious action of UV and X rays [1]. In therapy, flavones are used under different medicine forms: tablets, injection solutions, ointments, etc, [6]. Making up the yellow pigments in flowers, leaves, bark, and wood, flavones are widely used in analytic chemistry as colour and kellation reactants and in industry as colouring materials. In the food industry, they can be used as fat antioxidants [4]. Effects of different row spacing on dry petals yield, with directly implication of active principels, are presented in this paper. At Banat area condition, sowing optimum density was 70 pl/m^2, determinated to obtain the highest content of polyphenols and flavones.

MATERIALS AND METHODS

Total antioxidasing capacity is determined through the FRAP method (Ferric reducing ability of plasma), a method developed initially to measure antioxidant capacity of plasma. It is based on the capacity of reducing ferric ions to ferrous ions which, in an acid medium, form a complex coloured with tripyridyl triazine (TPZ), with maximum absorption at 593 nm. Antioxidasing capacity is directly proportional with the amount of ferrous ions formed, which can be determined on the ground of a sampling curve using samples of ferrous ion known concentrations.

Reagents: All chemicals and reagents were analytical grade purchased from Sigma, Merck or Fluka.acetate buffer pH: 3.6 (3.1 g sodium acetate x 3 H_2O and 16 ml CH_3COOH conc. In 1 l water TPTZ solution: 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 1 l HCl 40mM$FeCl_3$ solution: 20Mm $FeCl_3$ x $6H_2O$ in distilled water. Aqueous solution of know Fe (II) concentration was used for calibration. 2.5 ml FRAP reagent and 0.5 ml plant extract was heating at 37^{0}C, 4 minute and monitoring at 593 nm, 1 cm lights path. Calculation was done using the calibration curve. Total antioxidant capacity is directly proportional with the amount of ferrous ions formed.

Polyphenols are antioxidasing substances that can be found in considerable amounts in plant products. From a chemical point of view, they are aromatic compounds with several hydroxyl groups on the aromatic nucleus, which confers them redox properties, as they can be oxidised by the Folin Ciocalteu reactant with the formation of a bluish coloration with maximum absorption at 750 nm. Reagents: Na_2CO_3 7.5% solution, Folin Ciocalteu reagent, methanol/water (80:20, v/v).

Folin Ciocalteu reagent: 100 g Na_2WO_4 x $2H_20$ and 25 g Na_2Mo_4 x $2H_20$ was dissolved in 700 ml distilled water. 50 ml H_3PO_4 85% and 100 ml HCl 37% was added and the solution was boiling 10 hours by reflux. 150 g Li_2SO_4 X H_2O and some bromide drops was added and boiled 10 min. The solution is completed with water to 1000 ml. 10 ml sample extract was stirred 30 min with 10 ml methanol: water (80:20, v/v), the mixture is centrifuge 15 min. The supernatant, which content the polyphenols was analysed with 10 ml Folin Ciocalteu reagent and 9 ml Na_2CO_3 7.5% solution. The solution was stored 2 hours and the polyphenols are detected at 750 nm. Calibration curve is prepared from gallic acid standard mixed with methanol: water (80:20, v/v) within the concentration interval 25-500 mg/l gallic acid. The results are expressed as grams of gallic acid equivalents/100 g sample.

Sampling curve is prepared from gallic acid standard mixed with methanol: water (80:20, v/v) within the concentration interval 25-500 mg/l gallic acid.

The experience is at the Experimental Didactic Station of the Agricultural and Veterinary University of the Banat, Timişoara, on a chernozem. The climatic conditions of the studied years were favourable for the marigold production. The temperature before the harvest was high and facilitated the accumulation of active elements. From the point of view of rainfalls during 2006-2008, there were different values between the years, but their influence was positive. Fertilizing the crop was done evenly with $N_{15}P_{15}K_{15,}$ on autumn (during the primary tillage), in both years was applied 100 kg/ha and in spring (before sowing) 100 kg/ha urea (46% N). It was a monofactorial experience with density variation: V1 = 30 pl/m^2; V2 = 45 pl/m^2; V3 = 60pl/m^2; V4 = 70 pl/m^2. Biological material used was the Petrana cultivar, from the Research Institute Moara Domneasca. Sowing time is March. We use about 6 kg per ha. It can also be sowed it gradually, so that we can harvest it until October. In the field, we made measurements with the metrical frame in order to establish density.

Harvesting the inflorescences was done gradually, following blooming time and duration of blooming, average probe 100g "*Flores Calendulae cum receptaculis"*.

RESULTS

Petals is the most important herbal drugs of marigold because of content of active compounds. Significant differences were between row spacing of 30 pl/m^2 and 70 pl/m^2. We can also say that a density of 60 plants per m^2 also led to important yield increase. Figure 1, shows that sowing by 70 pl/m^2 led to an increase in dried inflorescence yield with 155 kg/ha compared to the control witch ensured an inflorescence yield distinctly significantly positive wers mass is important quality parameters. The lowest results in dried inflorescence yield oil yield were obtained on 30 pl/m^2 density (947 Kg/ha). A low result was obtained also on 45 pl/m^2 density: 995 kg/ha.

Figure 2 shows that sowing by 70 pl/m^2 led to an increase in polyphenol content from 0.288 g (%) to 0.308 g (%) with 7%. A low result was obtained on 30 pl/m^2 density: 0.288 g (%).

In Figure 3 we can see that increasing the density we increase also the flavones content.

From those two experiences we can observe that the sowing density has great influence on flavones content but also on polyphenol content.

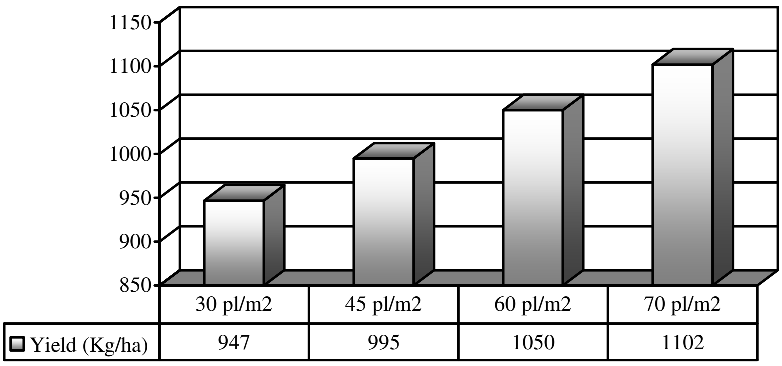

Figure 1. Variation of Dried inflorescence yield (kg/ha) obtain in Timişoara (Romania) 2006 - 2008

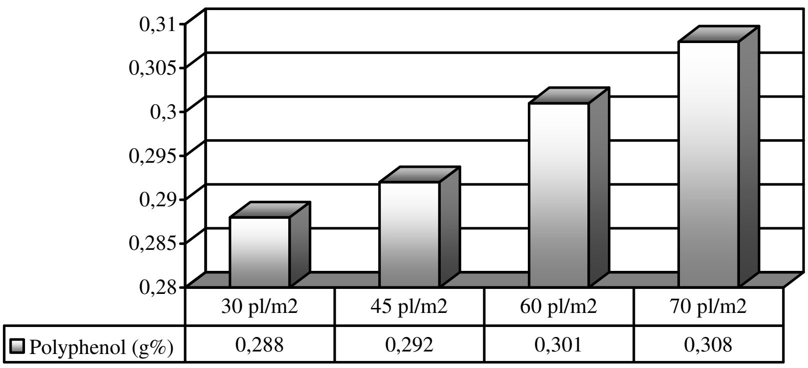

Figure 2. Polyphenol content (g%) variation depending on sowing density (2006 – 2008)

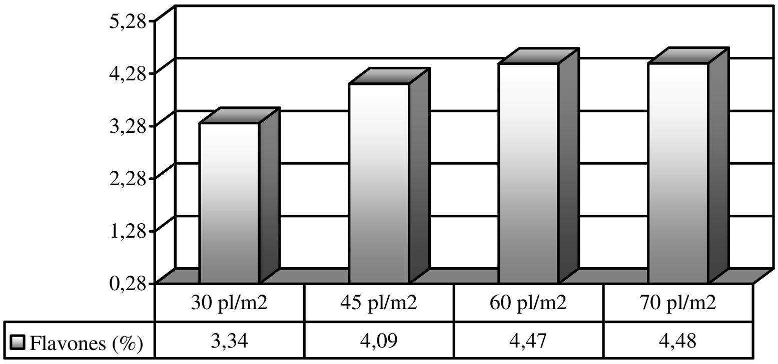

Figure 3. Flavones content (g%) variation depending on sowing density (2006 – 2008)

DISCUSSIONS

The highest yields in Romania were obtained when sowing density was 70 pl/m^2 [7].

At Cluj Napoca University good results was achieved at 50 cm distance between rows and 10 cm between plants on rows [5], and Evdochia Coiciu şi colab. recommended sowing in double rows at 15 cm between them and 50-60 cm between rows.

Increasing plants density show us that also the polyphenol content will be increased from 0.288 (g%) to 0.308 (g%) at 70 cm pl/m^2.

Also flavones content registered increasing the contain from 3.34 g% to 4.48 g% at maximum density (70 pl/m^2).

We can say that crop density can influence the total content of active principles, with directly influence of total yield.

The active principles of the analysed cultivar were significant influenced by the cultivation technology.

The quantity of the active principles is influenced by the sowing density and it is very important to take this fact in consideration for best result in oil yield.

Time of seeding, density, row distance, seeding intensity are factors who can influence the total yield and the raw materials quality.

The quantity of active ingredients from plant depending by ecological factors, crop technology, cultivars and also modality of primary and secondary processing [10].

In plant populations of the Marigold controlling and compensatory mechanisms such as thickening, thinning and stratification are at work being closer related to the ecological limits of the environment.

In substance, they appear to be conclusive in determining the total biomass, plant size, age structure, productivity. Many relationships have to be taken into account in optimizing the structure and architecture of the Marigold copses in order to acieve the maximum yield of flowers. As for density, the highest yield was in the variant sowed with70 pl/m^2, [8]

At Banat area condition, sowing optimum density was 70 pl/m^2, determinate to obtain the highest content of polyphenols and flavones. Marigold is one of the most values medicinal plants and it needs to be proposed for a larger area of cultivation. Cultivating this species allows agriculturists to get quality yields with high content of active principles that result in considerable incomes.

As many others medicinal plants, *Calendula officinalis* L.can bring an important profit to Romanian agriculture if is well cultivated.

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