# ALANTOLACTONE PROFILE IN *TAGETES ERECTA* AERIAL PARTS

## ALANTOLACTONA ÎN TULPINA, FRUNZELE ŞI INFLORESCENŢELE DE *TAGETES ERECTA*

***MIRCEA (ARSENE) Cristina[1,2], CIOANCĂ Oana[2], IVĂNESCU Bianca[2] , DRAGHIA Lucia[3], HĂNCIANU Monica[2]***
e-mail: oanacioanca@gmail.com

***Abstract****. The genus Tagetes (Asteraceae) comprises species with a wide arrays of uses. Previous studies have been focused on the distribution of flavonoids in the genus but little has been done on the identification of the sesquiterpenlactones in the medicinal, cosmetic and aromatic species. The significance of the distribution and accumulation of this compound thorough the plant is not yet clear. In the current study, the alantolactone content of aerial parts of Tagetes erecta was investigated, during budding and full flowering stages. The TLC and HPLC methods confirmed the presence of alantolactones, greater in budding stage sampes than in full flowering ones (0.2309 µg % in buds, 0.5097 µg % in leaves). The fertilized plant inflorescences contain greater amounts of studied metabolites than unfertilized plants. The leaves content is not influenced by fertilization. The presence of this metabolite alerts to the allergenic potential of plant, especially in budding stage.*
**Key words**: sesquiterpenlactones, TLC, HPLC

***Rezumat.*** *Genul Tagetes cuprinde specii cu o arie largă de utilizări. Studiile anterioare s-au concentrat în special pe distribuţia flavonoidelor în cadrul genului dar puţine studii se referă la identificarea sescviterpenlactonelor în speciile vegetale medicinale, aromatice şi cu utilizare în cosmetică. Semnificaţia distribuţiei şi acumulării acestor compuşi în plante nu este clară. În studiul prezent s-a investigat conţinutul de alantolactonă în părţile aeriene de Tagetes erecta, în fenofazele de boboc şi înlorire deplină. Metodele CSS şi HPLC au confirmat prezenţa alantolactonei, mai mare în fenofaza de boboc decât cea de înflorire deplină (0,2309 µg % în boboci şi 0,5097 µg % în frunze). Inflorescenţele plantelor fertilizate conţin mai multă alantolactonă decât plantele nefertilizate. Alantolactona din frunze nu este influenţată de fertilizare. Prezenţa acestui metabolit alertează asupra potenţialului alergenic al plantei, în special în fenofaza de boboc.*
**Cuvinte cheie:** sescviterpenlactona, CSS, HPLC

## INTRODUCTION

*Tagetes erecta* L. is a medicinal and ornamental plant. It has a wide array of uses, including cosmetics. Phytochemical studies of its different parts have results in

[1]SC ARA MANAGEMENT SA, Romania
[2]University of Medicine and Pharmacy Iasi, Romania
[3]University of Agricultural Science and Veterinary Medicine "Ion Ionescu de la Brad" in Iasi, Romania

the isolation of various chemical constituents such as thiophenes, flavonoids, carotenoids, triterpenoids (Devika and Justin, 2014).

The occurence of sesqiuterpenlactones (SQL) in many species of the *Asteraceae* is well known (Wagner and Bladt, 2001). It was found that these secondary metabolites occured in different concentration in all plant tissues (Mircea (Arsene) *et al.,* 2015). Alantolactone is a SQL with proven allergenic potential (Stampf *et al.,* 1982, Warshaw and Zug, 1996) and information regarding the presence of alantolactone in *Tagetes erecta* is still very limited. It is known that biosynthesis and concentration of secondary metabolites of plants depend on growing sites, climate conditions, cultural practices, vegetation phases and on cultivar and specific organs of the plant (Pljevljakušić *et al*., 2014, Ormeño and Fernandez, 2012, Ibrahim *et al.,* 2011).

The current article describes the alantolactone profile in fertilized and unfertilized plants, in different stage of development. Authentication of alantolactone was carried out by TLC (thin layer cromatography) and HPLC analysis .

## MATERIALS AND METHODS

Local conditions. The research was conducted at the Floricultural Department of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iasi, in the 2013-2015 cultivation years. Soil parameters and climatic conditions were evaluated.

Cultivation trial. The experiment was performed as plot design based on randomised complete blocks with three replications. *Tagetes erecta* plants were studied related to their macromorphology and alantolactone profile, in two nutritional statuses: local (V1) and enriched soil conditions (V2).

Seedlings of marigold were planted in the field at the beginning of May, at a density rate of 16 plants $m^{-2}$. The chosen fertilizer was osmocote-type, ecofriendly and 5-6 months availability; NPK (Mg): 15-10-12(2), microelements B, Cu, Fe, Mn, Zn, supporting ornamental growth and development with easy maintenance. The product was administered in 75g $m^{-2}$ just before planting in the first year and at the start of vegetative growth in the next years. Biometric parameters measured were: height and width of plant, number of principal ramifications per plant, number and diameter of inflorescences per plant. Ten plants were randomly selected from each plot for all parameters evaluated and the average was calculated in both nutritional statuses. Statistical significance between mean values was assessed through classic statistical calculations: significance of differences between the variants using LSD test. The variants were compared with their average. Stems and leaves (together) and inflorescences were studied for aforementioned chemical compounds content in two phenophases (budding stage and full flowering stage), in both nutritional statuses.

Chemicals and phytochemical analysis. *Chemicals* - All chemicals used in the present research were purchased from Sigma Aldrich (Germany). Stock solutions were prepared in HPLC grade methanol and stored at 4ºC until the finalization of the tests. All solvents were of analytical grade.

*Extraction methods* - For TLC evaluation dried plant material was extracted twice with ethanol 70% for 30 minutes under reflux (DER=1:10 g/mL) on a thermostatic water bath. An aliquot of 50 mL from each extract was concentrated using a rotary evaporator (Buchi R 210, Switzerland) to remove the solvent. The residues were dried and stored at 4°C for analysis. For HPLC alantolactone evaluation, the test solutions were obtained in acetonitrile R (1,0 g powdered drug in 3mL solvent,

three times, sonicated for 15 minutes). The filtrates were reunited and brought to 10mL in a volumetric flask.

Vegetal samples were noted as follow:

V1f1 – buds from unfertilized plants (budding stage);
V2f1 – buds from fertilized plants (budding stage);
V1f2 – inflorescences from unfertilized plants (full flowering stage);
V2f2 – inflorescences from fertilized plants (full flowering stage);
V1-1 – leaves and stems from unfertilized plants (budding stage);
V2-1 – leaves and stems from fertilized plants (budding stage);
V1-2 – leaves and stems from unfertilized plants (full flowering stage stage);
V2-2 – leaves and stems from fertilized plants (full flowering stage stage).
Standards used are noted in TLC chromatograms with: Ala – alantolactone;

*Terpenoids pattern evaluation by TLC.* In the present study were used: Standard compound: alantolactone, 12 µL; Solvents: hexane - ether (25:75, v/v); Detection: Zimmermann reagent, VIS evaluation.

The TLC plates were sprayed with 10mL of the reagent, heated at at 100°C for 5 min, and then evaluated in VIS.

*RP-LC-DAD analysis of sesquiterpene lactones (alantolactone).* A Thermo-Fischer UltiMate 3000 system coupled with DAD detector was used to assess the profile sesquiterpene lactones. The working conditions were: Accucore XL-C18 column (4.6 x 150 mm, 4 µm); column temperature: 34°C; detection wavelength was set at 225 nm and the flow rate was 1,2 mL/min. The mobile phase consisting of two eluents as A (water) and B (methanol) used the following linear gradient elution: 0-3 min 38%B; 3-20 min 45%B; 20-30 min 45% B; 30-55 min 55%B; 55-57 min 100%B; 70 min 100%B; 90 min 38%B. As standard, alantolactone was used in amount of 20 µL of a 5mg/mL solution. Samples UV spectra registered at 225nm were automatically compared by Chromeleon 7.2 software and the concentration was expressed as % of the standard's aria/concentration.

## RESULTS AND DISCUSSION

The fertilization increases the plant height, plant width, number of principle ramifications and number of inflorescences per plant, and also diameter of inflorescences. Distinctly positive differences were recorded in three parameters (tab. 1) and very positive significance was registered for plant width.

*Table 1*

***Tagetes erecta:* the influence of fertilisation on morphological parameters**

| Treat-men | Plant height (cm) | Plant width (cm) | Number of principle ramifications/ plant | Number of inflorescences/ plant | Diameter of inflorescences (cm) |
|---|---|---|---|---|---|
| **$V_1$** | $37.00^{00}$ | $30.00^{000}$ | $15.00^{00}$ | $10.28^{00}$ | $4.8^{ns}$ |
| **$V_2$** | $41.00^{xx}$ | $35.00^{xxx}$ | $21.00^{xx}$ | $12.15^{xx}$ | $5.9^{ns}$ |
| **Average** | 39.00 | 32.50 | 18.00 | 11.22 | 5.35 |
| LSD 5% | 0.74 | 0.25 | 1.08 | 0.39 | 1.55 |
| LSD 1% | 1.72 | 0.57 | 2.5 | 0.9 | 3.58 |
| LSD 0,1% | 5.47 | 1.82 | 7.95 | 2.87 | 11.39 |

*Note*: **oo/xx** = distinctly negative/positive significance;
**ooo/xxx** = very negative/positive significance; **ns** = not significant

The TLC general overview indicated that alantolactone (violet grey zones, Rf 0.74) is present in closed inflorescences of young plants (budding stage), in both nutritional statuses (fig. 1).

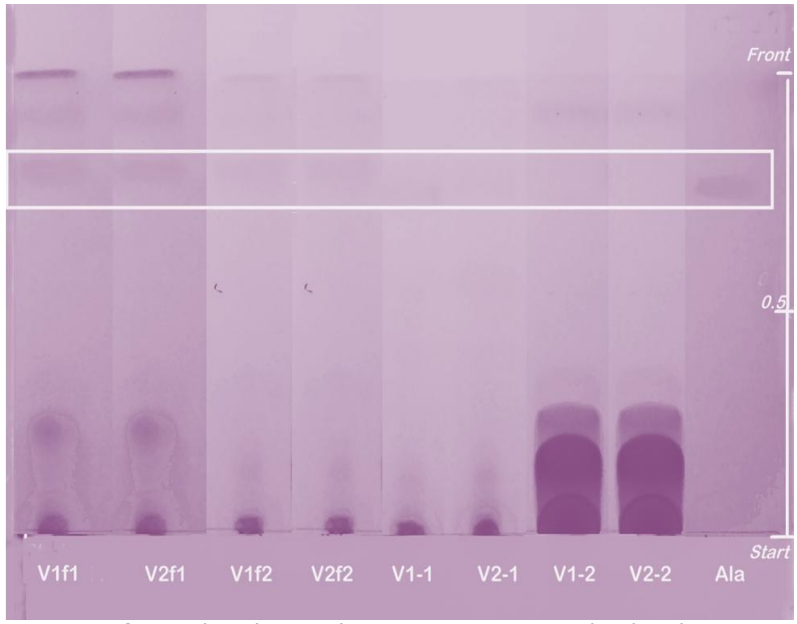


**Fig. 1.** Alantolactone in *Tagetes erecta* samples (TLC)

The HPLC analysis confirmed the presence of alantolactone (fig. 2). Comparative results obtained (TLC/HPLC) are tabulated in table 2.

Semiquantitative quantification showed that alantolactone is present at low levels (some micrograms at 100 gr dried plant) in buds and open inflorescences and in stems and leaves of budding stage plants. The fertilisation increases the alantolactone content of inflorescences.

Alantolactone represents a proven allergen and its presence in plants demonstrate that marigold is potentially allergenic.

*Table 2*

**Qualitative and semiquantitative identification of alantolactone in *Tagetes erecta***

| Method | Buds / Open inflorescences | | | | Stems and leaves | | | |
|---|---|---|---|---|---|---|---|---|
| | V1f1 | V1f2 | V2f1 | V2f2 | V1-1 | V1-2 | V2-1 | V2-2 |
| TLC | + | u | + | u | u | – | u | – |
| HPLC | 0,2309 | 0,0729 | 0,3009 | 0,2459 | 0,5097 | – | 0,5219 | – |

*Note: **u** = traces; **+** = present; **–** = not identifiable.*

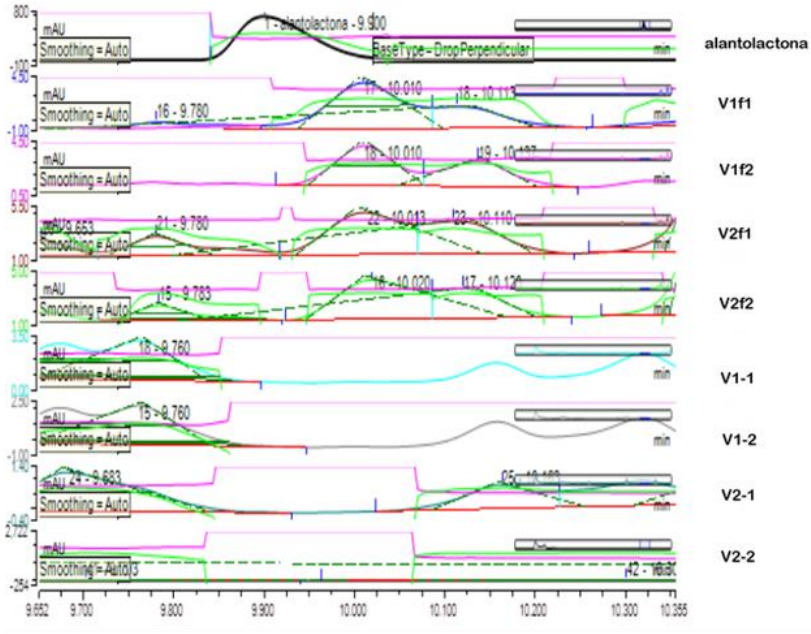


**Fig. 2.** HPLC alantolactone identification in *Tagetes erecta* samples

## CONCLUSIONS

Fertilisation with controlled release fertilizer intensifies plant growth and ramification, also increases the number of inflorescences per plant. The alantolactone content is greater in leaves and stems of young plants comparative with mature ones. Inflorescences contain alantolactone in all development stages and fertilised statuses.

Other studies are needed to complete the terpenoid chemical profile of this ornamental.

***Acknowledgments:*** *This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

### REFERENCES

1. **Devika R., Justin K., 2014** – *Screening and evaluation of bioactive components of Tagetes erecta L. by GC-MS analysis*. Asian Journal Of Pharmaceutical And Clinical Research, vol. 7, Suppl. 2, p.58-60.
2. **Ibrahim MH., Jaafar HZ., Rahmat A., Rahman ZA., 2011** - *Effects of nitrogen fertilization on synthesis of primary and secondary metabolites in three varieties of kacip Fatimah (Labisia pumila Blume).* International Journal of Molecular Sciences, 12, p.5238-5254.

3. **Mircea (Arsene) Cristina Cerasela, Cioancă Oana, Draghia Lucia, Hănceanu Monica, 2015 -** *Morphological characteristics, phenolic and terpenoid profiles in garden Chrysanthemum grown in different nutritional conditions*. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, vol. 43, nr. 2, p. 371-379.
4. **Mircea (Arsene) Cristina Cerasela, Cioancă Oana, Ivănescu Bianca, Draghia Lucia, Hănceanu Monica, 2015** - *The influence of fertilisation on the ornamental characters and on the alantolactone and triterpenic acid profiles in Echinacea purpurea*. Lucrări Stiințifice Seria Horticultură, vol.58, nr.2, pp. 261-266.
5. **Ormeño E., Fernandez C., 2012** - *Effect of Soil Nutrient on Production and Diversity of Volatile Terpenoids from Plants*. Current Bioactive Compounds, nr.8, p.71-79.
6. **Pljevljakušić D., Janković T., Jelačić S., Novaković M., Menković N., Beatović D., Dajić-Stevanović Z., 2014** - *Morphological and chemical characterization of Arnica montana L. under different cultivation models*. Industrial Crops and Products, 52, p.233-244.
7. **Stampf JL., Benezra C., Klecak G,. Geleick H., Schultz KH., Hausen B., 1982** - *The sensitizing capacity of helenin and of two of its main constituents, the sesquiterpene lactone alantolactone and isoalantolactone: a comparison of epicutaneous and intradermal sensitizing methods in different strains of guinea pig.* Contact Dermatitis, 8 (1), p.16-24.
8. **Wagner H., Bladt S., 2001** - *Plant drug analyses-a thin layer cromathography atlas*, 2nd edition, Springer, Berlin, 369p.
9. **Warshaw EM., Zug KA., 1996** - *Sesquiterpene lactone allergy*. Am J Contact Dermat, 7, p.1-23.