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CONSIDERATIONS ON THE HISTO-ANATOMICAL STUDY OF THE LEAVES OF CYNARA SCOLYMUS L. TREATED WITH METHYLTHIOPHANATE (TOPSIN M)

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Keywords: *Cynara scolymus L, thiophanat-methyl, histo-anatomical modifications, leaf structure.*

INTRODUCTION

The therapeutically importance of the artichoke being unanimously recognized (Stănescu Ursula, 2004) its introduction in intensive cultures require, in some cases, the application of certain phito-sanitary treatments (Mititiuc, 2000; Verze Maria, 2003), once known that the newly-created microclimate favourizes mass development of pathogenic agents such as: *Septoria scolymii* Pass., *Ascochyta cynarae* Maffei., *Ramularia cynarae* Sacc.(Săvulescu Alice, 1967), *Bremia lactucae* Regel.(Corda, Franceschini, Fiori, 1983). Thiophanate-methyl has quite a large action spectrum (on the *Septoria, Ramularia, Bremia, Sclerotinia* species included), the literature of the field providing several data on the successful application of such a fungicide, as well as of its main metabolite-carbendazime (Marras et all, 1983)

As most of the researches devoted to methyl-thiophanate have been mainly directed towards metabolism, toxicity or estimation of the maximum residual potential in the vegetal material (Marras şi colab., 1983), and considering the fact that, generally, pesticides induce morpho-anatomical modifications in the treated plants (Georgescu, Sanda, 1964; Niță Mihaela,. 2003; Aprotosoaie Clara, 2002), the present paper analyzes the possible histo-anatomical modifications that the fungicide and/or its metabolite might induce at the level of the vegetative organs – at the level of the leaf, especially (the *Cynarae folium* pharmaceutical product) – versus the untreated control sample, the structure of which has been described in several papers of vegetal anatomy (Solereder, 1899; Rácz, Pèter, Sebe, 1967; Rácz, Pèter, 1968; Metcalfe, Chalk, 1972; Napp-Zinn, 1974 Rugină, Toma, 1989; Toma, Rugină 1998).

MATERIALS AND METHODS

The material taken into study has been cultivated in the „Anastasie Fătu" Botanical Gardens of Iaşi in parallels whit the treated plants (Topsin in 0.1% concentration – as used in agriculture, and 0.4 % concentrated Topsin, respectively, which is the value recommended for other fungicides, similar to methyl thiophanate), a batch of untreated plants – the control – has been also involved. Fungicide's administration as a wettable powder, in doses of 1000 l/ha, was performed three times (at intervals of 7 and 10 days), in the moment in which the foliary system of the artichoke was already well developed, the basal rosette having 5-6 nomophyls. For the obtention of cross-sections, at different levels, through the leaf (petiole, limb), as well as of superficial sections, the vegetal material harvested twice, at 7 days after the second and, respectively, third treatment (July 23 and August 3) has been fixed and conserved in 70% ethanol, after which it has been processed according to the methods currently employed.

RESULTS AND DISCUSSION

The petiole (fig. 1-2). In the control sample (M), the contour of the cross-section (medium level) is V-shaped, with highly divergent and increasingly thinner arms. The abaxial side evidences 5-7 obtuse ribs, while the adaxial one – a large and deep ditch. The epidermis evidences iso-diametric cells, rare stomata, prominent on the inferior part, where the glandular and nonglandular trichomes (long, with a thin and flexuose terminal cell) are much more numerous; the short nonglandular trichomes, with an extremely wide basal cell, are very rare.

In the abaxial ribs (8-9 layers) and at the end of the arms, a thick belt of angular cholenchyma may be noticed while, between the ribs, the hypodermis is of the chlorenchymatic type. In the fundamental, homogenous parenchyma, there appear numerous conducting bundles (22-24), the big alternating with the small ones, all of them arranged as a double, large-open arch.

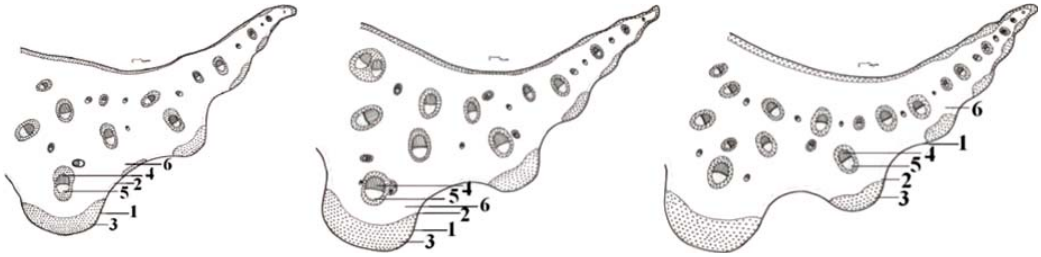


Fig. 1. Diagrams of cross-sections through the petiole, medium level, August 3; - 1 epidermis; 2- chlorenchyma; 3- cholenchyma; 4- xylem; 5- liber; 6- fundamental parenchyma.

Fig. 2. Cross-sections, petiole, medium level, conducting bundle (Oc. 10x Ob.10), August 3.

With the exception of the very small ones, each conducting bundle shows, at both poles, a thick girdle of mechanical elements, with moderately-thick, yet non-lignified walls, resembling a transitive cholenchyma. At the xylemic pole of the large bundles, in the mechanical tissue, two very small conducting bundles of closed collateral type may be noticed; in the other bundles, of open collateral type, the tracheogenesis process is not completed yet. Each conducting bundle is surrounded by a parenchymatic pod at the level of which, in the xylemic part, 5 secreting channels are visible.

Following treatments with 0.1% and 0,4 % Topsin M70, the petioles thickness increases considerably, the cholenchyma girdles show several (12-13) layers, and the degree of the cellular walls' thickening increases, too. At the level of the epidermis, the stomata are highly prominent on the inferior side, while the glandular and nonglandular trichomes are more numerous on the surface unit.

The conducting fascicles, much larger in size, are more numerous (46-48); generally after treatments, the number of small conducting bundles increases; cholenchymatization is much more intensive at the bundles' poles, the mechanical tissue being here a typical angular cholenchyma; the xylem vessels are larger and more numerous, with thicker and more intensely lignified walls; the cambial activity is stimulated (3-4 layers), several xylem vessels being still immature. In the perifascicular pod, secretory ducts (1-2) do appear and, in front of the liber, their number (7 on the whole) is higher after the 0,1% TM treatment.

Table 1. Variation - under treatment with 0.1% and 0.4 % Topsin M - of some numerical indices in the petiole (medium level) of the *Cymara scolymus* leaves taken over on August, 3

Variant	Thickness (μm)	No. of cholenchyma layers	No. of conducting bundles	Diameter of the xylem vessels (μm)
Cy. sc. M	4100-4300 7300-7500 (x2)	8-9	34-37	(30) 50-60
Cy. sc. TM 0,1 %	4500-5300 7600-7800 (x2)	12-13	35-40 (42)	(50) 70-90
Cy. sc. TM 0,4 %	4700-5500 7800-8000 (x2)	10-11	46-48	(50)70- 90

The limb (fig. 3-7). In the control sample (M), the median nervure is prominent on the inferior side, where the long and fine nonglandular trichomes, as well as the glandular ones, whit the cells

of the secretory gland in tiers, submerged towards the basis of the hair, are more numerous on the unit of surface. In the abaxial ribs (3), girdles of angular cholenchyma with 3-4 layers may be observed while, at the adaxial side a cholenchyma ridge appears. The median nervure shows 1-2 aeriferous cavities and 5-6 conducting bundles, all of them evidencing a mechanical tissue at both poles. The limb as such shows an epidermis with small cells, slightly elongated tangentially on the inferior part and larger ones, visibly elongated tangentially, on the superior part. The hairs are much more numerous in the inferior epidermis (the secretory ones occurring in excavations through the epidermis); equally numerous are the stomata, although they are present on both sides of the limb (aphistomatically). The mesophyll (5-6 layers) is differentiated into untypical, bi-layered pallisade parenchyma on the superior part and pluri-stratified spongy parenchyma on the inferior one (heterofacial bifacial limb). The pallisade parenchyma with relatively law cells (only two times higher then large), with straight or slightly curled lateral walls, represents about 45-50% of the mesophyll's thickness (fig. 5).

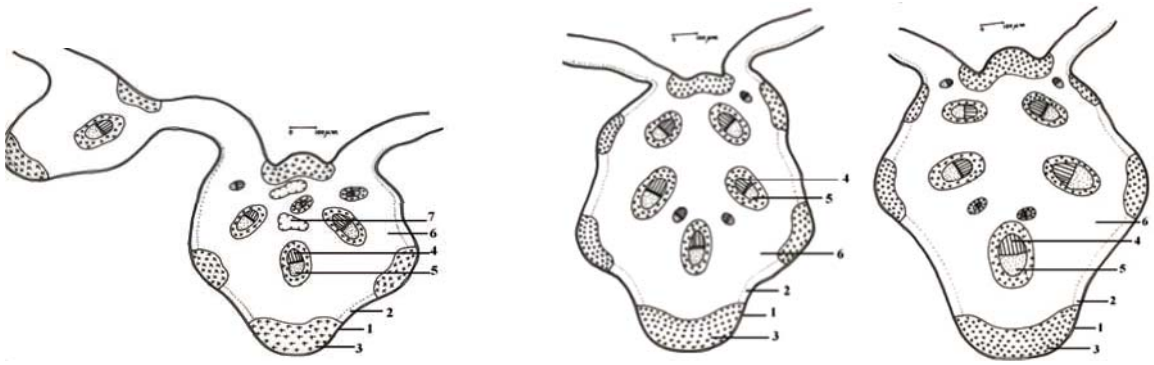


Fig. 3**.** Diagrams of cross-sections, limb, medium level, Aug. 3;-1 epidermis; 2chlorenchyma; 3- cholenchyma; 4- xylem; 5- liber; 6- fundamental parenchyma; 7- aeriferous gap.

As a result of treatments (0.1%, 0.4 % TM), the nervures become much thicker, especially in the anterior-posterior plane, the auriferous cavity is absent, while the more compact fundamental parenchyma includes several conducting bundles (7-8) 5 abaxial ribes are visible, each having 5-6 layers of cells with a higher cholenchymatization degree. The limb is thicker, which occurs especially at the expense of the pallisade parenchyma (cells' growing in length being stimulated), which is three-layered, representing 65-70% (in 0.1% TM treatments) and, respectively, 75-80% (in 0.4% TM treatments) of the mesophyll's thickness.

At the level of the superior epidermis, the walls are slightly thicker, the glandular and nonglandular trichomes are more numerous per unit of surface, the bi-seriated secretory ones prevailing between the secondary nervures and towards the margin of the limb; also here, short nonglandular trichomes, with a much larger basis and thickened walls are visible, as well, in the upper epidermis.

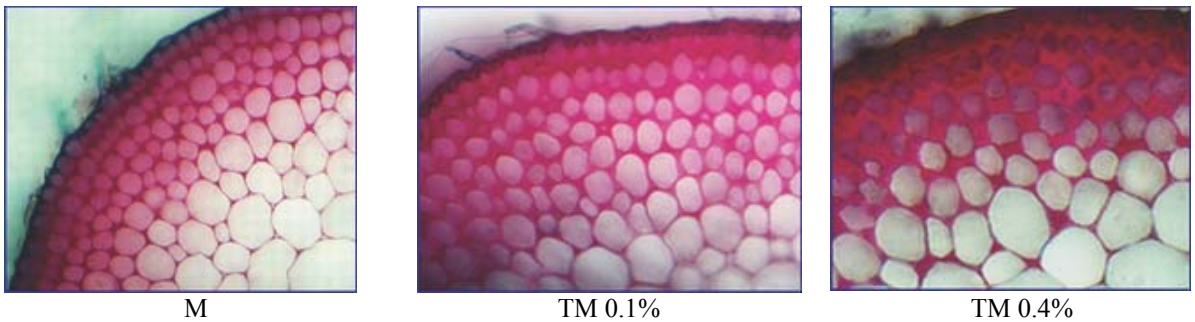
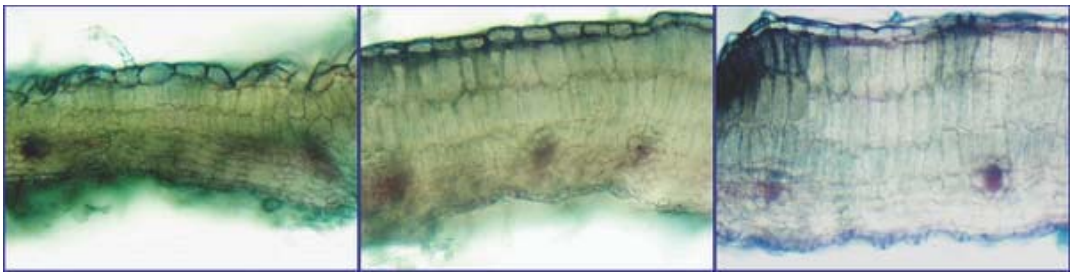


Fig. 4. Cross-sections through the limb, medium level, cholenchyma, foto: (Oc. 10x Ob. 20), August, 3.

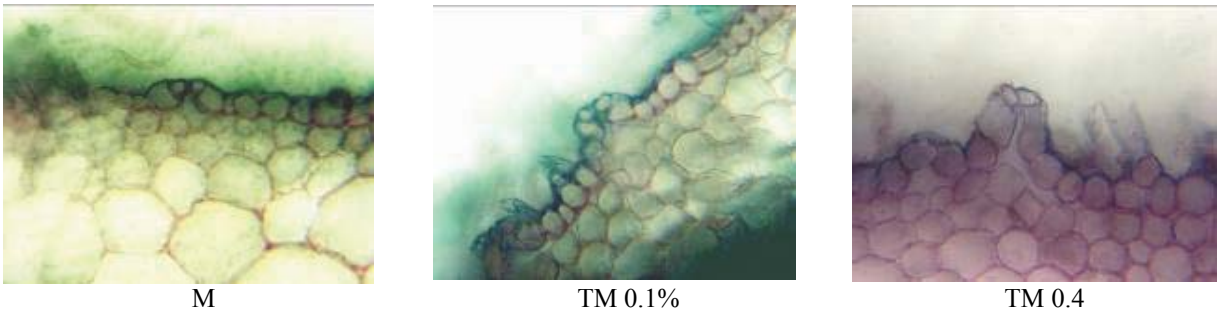


M TM 0.1% TM 0.4

Fig. 5. Cross-sections through the limb, medium level, mesophyll foto: (Oc.10x Ob. 20), August, 3.

Table 2. Variation under treatment with 0.1% and 0.4 % Topsin M of some numerical indices in the limb (median nervure, medium level) of the *Cymara scolymus* leaves taken over on August, 3

Variant	Diameter of the median nervure (μm)	No. of cholenchyma layers	No. of conducting bundles	No. of layers of the limb	Thickness of the limb (μm)	Thickness of the pallisade (μm)
Cy. sc. M	2100/2500	3-4	5-6 (7)	6-7	250-270	130-140
Cy. sc. TM 0,1 %	2400/3900	6-7	6-8	7-8	290-300	180-200
Cy. sc. TM 0,4 %	3200/5100	5-6	7-9	8-9	290-320	210-240



M TM 0.1% TM 0.4

Fig. 6. Cross-sections through the limb, medium level, stomata, foto: (Oc.10x Ob. 40), Julay, 23

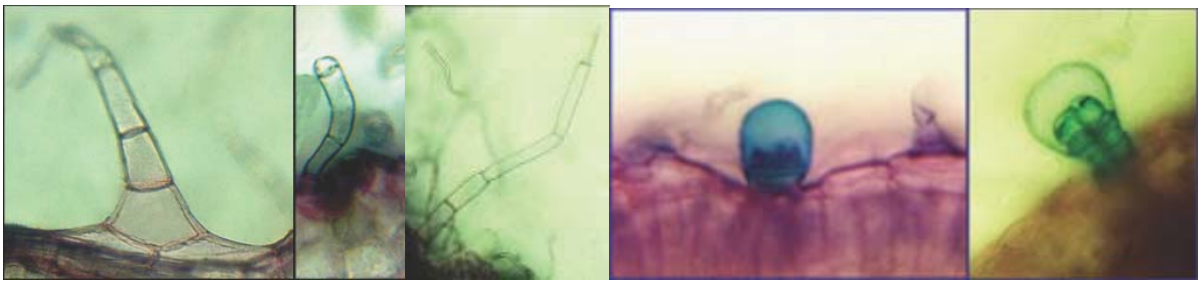
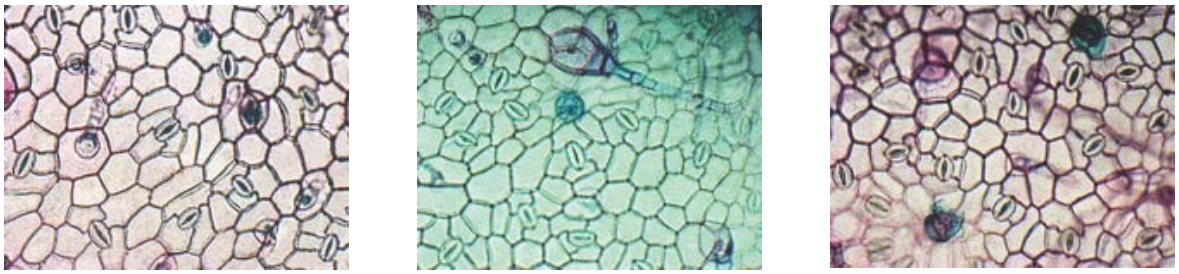


Fig. 7. Cynara scolymus: nonglandular trichomes, (a, b, c) and glandular trichomes: uniseriated- d, biseriated- e

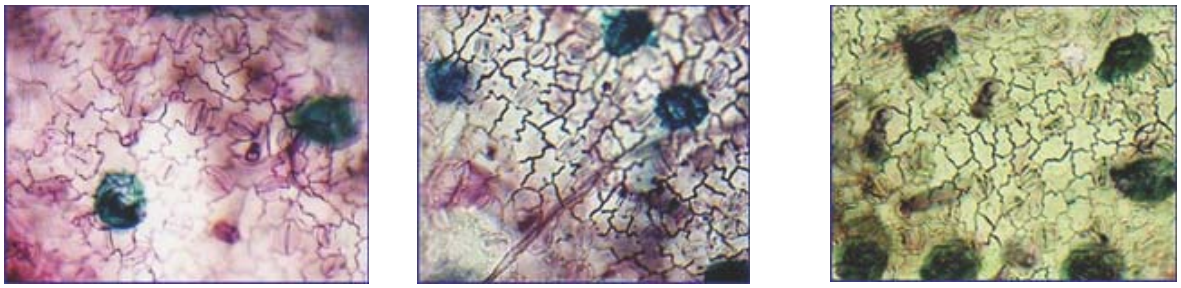
Superficial section epidermis (fig. 8, 9). In the control sample, the superior epidermis evidences polygonal cells with straight lateral walls; amonocytic-type stomata; pluricellular nonglandular trichomes of various thickness, with a very long flexuose, terminal cell, some of them being shorter, pluricellular, uniseriated, with the basal cell evidencing thickened walls;the uni- or biseriated secretory trichomes are very rare. The inferior epidermis shows irregularly-shaped cells, with winding lateral walls; the stomata are more numerous on the surface unit; the nonglandular trichomes (of both categories) are numerous and only very rarely biseriated. The number of cells per unit of surface is more reduced in the superior epidermis than in the inferior one.

The treatments (0.1%, 0.4 % TM) result in more numerous epidermic cells on the unit of surface, being therefore smaller; the number of stomata increases significantly, in parallels with a reduction in the size of the annex cells. The glandular and nonglandular trichomes are more numerous on the surface unit.



M TM 0.1% TM 0.4%

Fig.8. Superficial sections of the limb (superior epidermis) medium level, August, 3



M TM 0.1% TM 0.4%

Fig.9. Superficial sections of the limb (inferior epidermis) medium level, August, 3

Table 3. Numerical data on the epidermis of the *Cymara scolymus* taken over on August, 3 (per unit of 10x40 microscopic field)

Sample	Superior epidermis		Inferior epidermis	
	No. of cells	No. of stomata	No. of cells	No. of stomata
Cy. sc. M	35	4	115	26
Cy. sc. TM 0,1 %	38	5	120	29
Cy. sc. TM 0,4 %	52	7	126	32

SUMMARY

The purpose of this study was to investigate the histo-anatomical modifications of the leaves of *Cynara scolymus* L treated with thiophanate-methyl, compared with the untreated sample. There were three applications and two variants of treatment: 0.1 %, Topsin M70, a concentration used in agriculture and 0.4 % Topsin M70, for observing whether the limits of concentration between the modifications induced by thiophanate-methyl remain acceptable for the plant. Cross sections through the petiole and limb (the officinal product *Cynarae folium*), made at different levels were used, together with superficial sections for the upper and lower epidermis. The modifications observed were rather quantitative than qualitative, the general picture showing that the development of leaves was obviously stimulated.

For the treated plants, the comparative study revealed the following aspects: the thickness of petiole increased, the hypodermic walls of collenchymas are more developed, more specialized conducting bundles appear, with a larger diameter of the xylem vessels, cambium layer's activity is more intensive; the form and dimensions of the median nervure are modified, the mesophyll tends to thicken, the pallisade parenchyma being much more developed; the glandular and nonglandular trichomes are more numerous per unit area of leaf surface, the epidemic cells are many but more smaller and stomata more numerous per unit area, with guarding cells of smaller dimensions.

CONCLUSIONS

The histo-anatomical modifications induced by the treatment with Topsin M are most frequently of quantitative type, the plants free from the negative effects of the pathogenic agents having still to face another stressing factor, namely the anti-fungi treatment, especially in concentrations of 0.4%, applied for establishing the concentration limits between which the modifications induced by the fungicide remain acceptable for the plant.

The general picture of the response reactions should differentiate, in a clear-cat manner, between the objective of a higher production, as a result of more reduced losses, and the one related to the direct influence of the substance upon the plant, when some parts of the phyto- sanitary product may have a stimulating effect, as actually asserted by the literature

of the field (Baicu 1972).

For the treated plants, the comparative study put into evidence the following aspects: petiole's thickness increases, cholenchymatization is more pronounced, more numerous conducting bundles, with larger xylem vessels, do appear, cambial activity is more intense; the median nervure modifies its shape and sizes, the mesophyll gets thicker, while the pallisade parenchyma is better developed; the glandular and nonglandular trichomes are more numerous on the unit of surface; the epidermic cells are numerous and smaller, while the stomata are more numerous on the unit of surface, the latter ones having annex cells of lower size.

REFERENCES

1. APROTOSOAIE CLARA and all., 2002 - *Changes of anatomical features of some Lamiaceae species under treatment with antifungal Topsin M.* -2nd Conference on medicinal and aromatic plants of southeast European countries, Chalkidiki, Greece, September 29 - October 3, 2002, Book of abstracts, 132.
2. BAICU T, 1972 - *Utilizarea rațională a preparatelor fitofarmaceutice*. Probleme Agricole, XXIV, 6: 44 – 51.
3. CORDA P., FRANCESCHINI A., FIORI M., 1983 - *Prove di lotta contro la „Peronospora" (Bremia lactucae Regel.) del carciofo (Cynara scolymus L.) in Sardegna.* La difesa delle piante, 1: 13 – 16.
4. GEORGESCU C. C., SANDA V., 1964 - *Considerații asupra modificărilor anatomice produse la frunzele de Nardus stricta L. prin tratament cu erbicide și îngrășăminte azotoase.* Stud.și cer. de biol., ser. Bot., 16 : 143 – 149.
5. MARRAS F. et all., 1983 - *Residui di fungicide benzimidazolici nel carciofo (Cynara scolymus L.)*. La difesa delle piante, 2: 67 – 74.
6. METCALFE, C. R., CHALK L., 1972 - *Anatomy of the Dicotyledons II.* Clarendon Press, Oxford: 783 – 804.
7. MITITIUC M., HATMAN M., FILIPESCU C., 2000 - *Bolile și dăunătorii plantelor medicinale și aromatice.* Ed. Univ. "Al. I. Cuza" Iași: 87-91.
8. NAPP-ZINN Kl., 1973, 1974 - *Anatomie des Blattes. II. Angiospermen. In: Handbuch der Pflanzenanatomie, VIII, A 1-2,* Gebrüder Borntraeger, Berlin, Stuttgart: 676, 887.
9. NIȚĂ MIHAELA, 2003 - *Structure of the vegetative organs of both normal and herbicide treated Cynodon dactylon (L.) Pers.* An.șt. Univ. "Al. I. Cuza"Iaşi, s. II, a, (Biol. veget.), 49: 19 – 24.
10. RÁCZ G., PÈTER H. M., 1968 - *Valoarea diagnostică a țesutului epidermic și identificarea frunzelor oficinale în Farmacopeea Română (ed. a VIII-a).* Farmacia, 16:435-440.
11. RÁCZ G., P ÈTER H. M., SEBE B., 1967 - *Tipurile de stomate la drogurile oficinale în Farmacopeea Română (ed. a VIII-a*). Rev. Medic., 13: 185-188.
12. RUGINĂ RODICA, TOMA C., 1989 - *Recherches histo-anatomiques sur quelques plantes médicinales de la famille des Composées.* An. Șt. Univ. "Al. I. Cuza" Iaşi. s. II, a, (Biol. veget.), 35: 15 -18.
13. SĂVULESCU ALICE, RAICU CRISTINA 1967 - *Paraziții plantelor medicinale și oleo – eterice cultivate în România și unele mijloace de combatere*. Stud. și cer. de Biol. ser. Bot., 19, 3: 211 – 213.
14. SOLEREDER H., 1899 - *Systematische Anatomie der Dicotyledonen.* Fr. Enke Verlag, Stuttgart: 515-528.
15. STĂNESCU URSULA, MIRON ANCA, HÂNCEANU MONICA, APROTOSOAIE CLARA, 2004 - *Plante medicinale de la A la Z.* I. Ed. U.M.F., "Gr. T. Popa", Iași: 169-173.
16. TOMA C., RUGINĂ RODICA, 1998 - *Anatomia plantelor medicinale - Atlas.* Ed. Acad. Rom.,Bucureşti: 109 -11.
17. UNGUREAN L., SĂNDOIU D. D., CIONTU C., MATEI E., 1990 - *Modificări morfo – anatomice la porumb sub influența alaclorului și a atrazinului.* Combaterea integrată a buruienilor, Târgoviște, 7: 233 – 244.
18. VERZEA MARIA et all., 2003. *Tehnologii de cultură la plantele medicinale și aromatice.* Ed. Orizonturi: 27 – 34.

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