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HISTO-ANATOMICAL, CYTOGENETIC, AND BIOCHEMICAL RESEARCHES ON THE *INULA* L. SPECIES FROM THE ROMANIAN FLORA

PHD. THESIS-ABSTRACT

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INTRODUCTION

The *Inula* L. genus is part of the *Asteraceae* family and comprises about 120 species, extended in the temperate area of Europe and Asia (Nyarady, 1964). In the Romanian flora there are 10 species, out of which, one has no longer been confirmed by scientific literature, older (Nyarady, 1964) or newer (Oprea, 2005). In the same time, six hybrids are cited (Nyarady, 1964) or 5 hybrids in the N-E of the country (Chifu et al., 2006).

Of all the *Inula* species growing in our country, *Inula helenium* L. Is considered a valuable medicinal plant (Perrot and Paris, 1971). In recent works of pharmacognosy and phytochemistry other speciees of *Inul* are mentioned: *Inula viscosa* (Nikolakaki and Christodoulakis 2004), *Inula germanica, Inula ensifolia, Inula britannica, Inula crithmoides, Inula conyza* (Perrot and Paris, 1971).

The anatomical structure of the *Inula* species has very little been studied. In the treateses of dycotyledonous plant anatomy (Metcalfe and Chalk, 1972) or of the angiosperms in general (Napp-Zinn, 1973, 1974), only the *Inula* genus is mentioned. In some atlases of medicinal plant microscopy (Terpilo, 1961; Toma and Rugina, 1998) only *Inula helenium* L. is given importance.

In the present thesis, a special attention is given to the histo-anatomical study of the vegetative organs of the *Inula* species, otherwise little studied, except *Inula helenium* which interested some authors.

The aim of thePhd thesisa was the histo-anatomical, cytogenetic and biochemical of some plant species belonging to the *Inula* genus from the North-East of Romania.

The objectives of the research:

- A comparative anatomy study on the *Inula* L. species from the Romanian flora, highlighting the traces with taxonomic value, and also some of anatomo-ecological order, insisting on the structure and locations of the secretory canals, of the tector hairs and of the secretory ones by the help of classical and modern microscopy (SEM);
- The characterization of the mitotic chromosomes of some *Inula* species;
- The qualitative and quantitative phytochemical analysis of the active principles of *Inula helenium* L by chromatogrphic methods (CSS) and their dosing by means of spectrometry.

The *Inula* L. genus is part of *Spermatophyta* division, the *Magnoliophytina* (*Angiospermae*) subdivision, the *Magnoliopsida* (*Dicotyledonatae*) class, subclass *Asteridae*, the *Asterales* (*Compositales*) order, the family: *Asteraceae* (*Compositae*), subfamily *Asteroideae* (*Tubuliflorae*), the *Inuleae* tribe (Ciocirlan, 2000).

The *Inula* genus comprises plant species with ligulated flowers and tube formed, of the same colour; this genus is a kin of *Senecio, Arnica, Tagetes, Tussilago* etc. (Stefan and Oprea, 2007).

All the 10 species of *Inula* in the present study are from the wild flora, as follows: *Inula helenium* L., *Inula oculus-christi* L., *Inula salicina* with ssp. *salicina* and ssp. *aspera* (Poir.) Hayek, *Inula ensifolia* L., *Inula hirta* L., *Inula bifrons* (Gou.) L., *Inula conyza* DC., *Inula spiraeifolia* L. and *Inula germanica* L.

***Inula helenium* L.** (orig.)

fig.1

***Inula britannica* L.** (orig.)

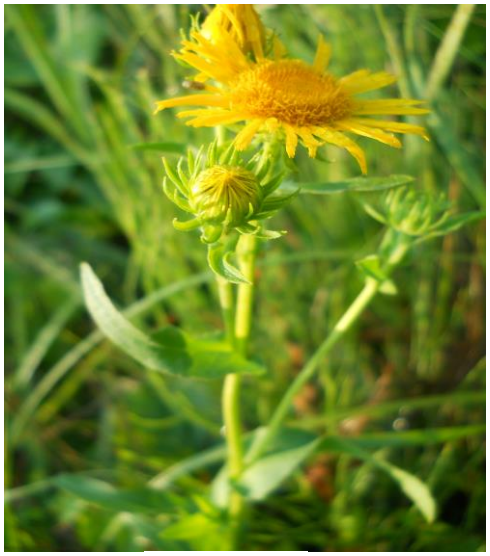

fig.2

***Inula oculus-christi* L.** (orig.)

fig.3

Inula salicina (http: // luirig. altervista.org / naturaitaliana)

fig.4

***Inula ensifolia* L.** (orig.)

fig.5

***Inula hirta* L.** (http: // luirig. altervista.org / naturaitaliana)

fig.6

***Inula bifrons* (Gou.) L.** (http: // luirig. altervista.org / naturaitaliana)

fig.7

***Inula conyza* DC.** (http: // luirig. altervista.org / naturaitaliana)

fig.8

***Inula germanica* L.** (orig.)

fig.9

***Inula spiraeifolia* L.** (http: // luirig. altervista.org / naturaitaliana)

fig.10

The general part has 3 chapters: The history of the researches regarding the structure and chemical composition of the ***Asteraceae*** species, of those belonging to the ***Inula*** genus, especially; The taxonomy of the ***Inula*** genus and the morphological characterization of the species; Materials and methods of research. The investigated material was collected from different locations in Moldavia and from the Danube Delta, including the natural reservations. The methods are the currently used ones in the researches of photonic and electronic microscopy, in the cytogenetic and biochemical researches (chromatography, spectrophotometry).

The special part has 4 chapters: The structure of the plantlets and mature plants vegetative organs (in the antesis stage); Micromorphological aspects of the leaf and fruit surface; The characterization of the mitotic chromosomes; The qualitative and quantitative determination of the active principles of ***Inula helenium*** (a species with medicinal value).

❑ The structure of the plantlets and mature plants vegetative organs

• The structure of the vegetative organs of *Inula* L. plantlets (drawings I, II)

After the analysis of the vegetative organs structure of the plantlets belonging to the 4 species studied we highlighted the following aspects:

The root presents rhizoderm with big cells, with all the cell walls thin; the external one is visibly bilging in case of *Inula ensifolia*. Here and there one may notice adsorbant hairs. The thickness of the cortical parenchyma varies: 7-8 strata of the meatic type for *Inula helenium*, 5 strata for *Inula ensifolia* and 4 strata for *Inula hirta* and *Inula britannica*. In the case of all the analyzed species, the endoderm presents Caspary thickenings in the radial cell walls. The stele is of several types: diarh at the *Inula ensifolia* plantlets, triarh, for the *Inula helenium* and *Inula hirta*, tetrarh for the *Inula britannica* plantlets. At the level of the central cylinder (of the tetrarh type) of *Inula britannica* there is a transfer to the secondary structure so that along with the 4 primary wooden fascicles there also appeared secondary wooden vessels.

The hypocotyl presents an epidermis with tangent or isodiametric prolongued cells (*Inula ensifolia*), with all the walls thin and with the exterior wall thicker than the others (*I. helenium, I. hirta, I. britannica*). Here and there, at the level of the epidermis there are already very numerous uniserial multicell tector hairs at *Inula hirta*. The number of the cortical strata differs: 10-12 for *Inula helenium*, 5-6 strata for *Inula ensifolia* and *Inula hirta*, 4-5 for *Inula britannica*. At the internal face of the crust one my notice **secretory canals** (*Inula helenium* and *Inula hirta*). The structure is primary in case of all species; with *Inula britannica*, the structure is intermediary between that of the root and the bladed. Those are visible as well as 4 free fascicles, and the woodeen vessels have irregular distribution, the ones of the organ axis being much smaller; among the wooden vessels with the walls moderately thickened and lignified there are cells of cellulose parenchyma.

The leaf. In the cros section, the foliar limb presents an outstanding median nervure on the inferior face, with a libero-wooden conducting fascicle, having mechanic fibres at both poles. The epidermis presents two types of **hairs**: **tector**, bicellular (*I. helenium*) and pluricellular, either thick and short (*I. helenium, I. hirta*), or long and thin (*I. hirta, I. ensifolia* and *I. britannica*); **secretory hairs** with a relatively long pedicel, formed of two lines of cells (biseriated) with bicellular gland (*I. helenium, I. hirta*). For all the plants we analyzed, the mesophill is thin, homogenous, of the lacunous type, so the limb has an isofacial bifacial structure.

fig.1

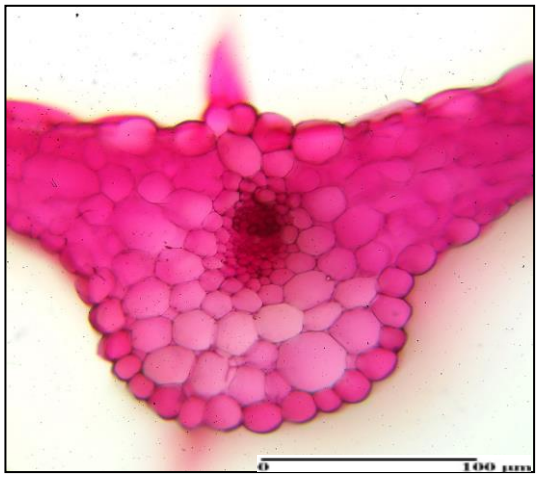

fig.2

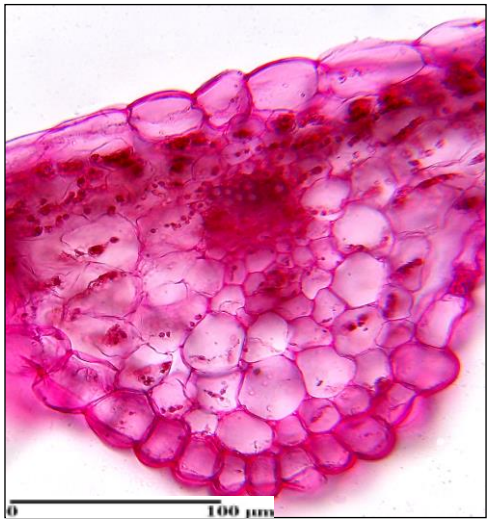

fig.3

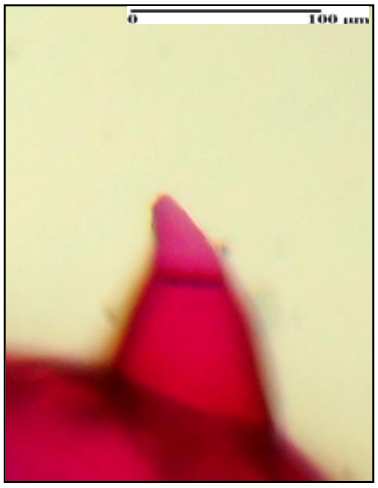

fig.4

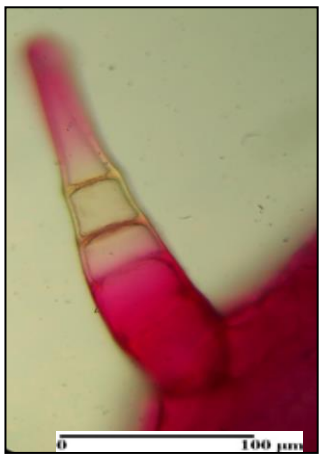

fig.5

fig.6

fig.7

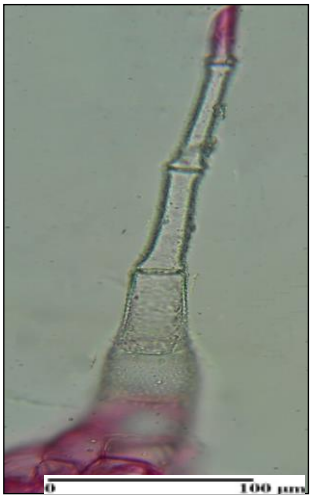

fig.8

Drawing I. Plantlets of *Inula*. Cross sections of the foliar limb- detail of the median nervure: *I. helenium* (fig. 1), *I. hirta* (fig. 2); *I. britannica* (fig. 3); pluricellular tector hairs: *I. helenium* (fig. 4, 5), *I. hirta* (fig. 6), *I. ensifolia* (fig. 7), *I. britannica* (fig. 8) (orig.)

fig.9

fig.10

fig.11

fig.12

fig.13

Drawing II. *Inula* plantlets. Cross sections through the foliar limb, secretory hairs: scheme (fig. 9), detail: *I. helenium* (fig. 10), *I. hirta* (fig. 11-13) (orig.)

- **The structure of the vegetative organs of mature *Inula* L. plants**

The results regarding the histo-anatomical structure of the vegetative organs of the *Inula* specie from the Romanian flora assert, on the one hand, the data contained by scientific literature (especially referring to *I. helenium*), and, on the other hand, they complete the same data as there very few titles referring to the anatomy of these taxons.

Terpilo (1961) analyzes the root structure of *Inula helenium* L., highlighting the following characteristics:

- the presence of an exterior epidermis;
- the presence of the secretory canals in the primary, secondary crust and at the level of the liber and the secondary wood;
- in parenchym cells of the root and rhizome there is inuline in the form of sphere shapes crystal.

Metcalfe and Chalk (1972) refer to some peculiarities of the *Inula* genus:

- the presence of anomocytic stomata;
- the frequency of uniserial pluricellular hairs, with the terminal cell very long, and the a very thick wall;
- the presence of secretory cavities in the underground parts.

Toma and Rugina (1998) investigated the vegetative organs (root, stem and leaf) of *Inula helenium*, stating the following:

- the root presents a typical secondary structure, as a result of the activity of the two lateral meristems: cambium and felogene; at itd lecel:
  - the liber presents riddled tubes, annex cells and a great quantity of amiliferous parenchym;
  - the wood is formed of vessels of different diameter, isolated or grouped;
  - in vecinity of the vessels there are cells with sphere shaped crystal of inuline;
  - the secretory canals are present in al the anatomical areas of the root.
- at the stem level:
  - the epidermis presents long secretory and tector hairs, a prezintă peri tectori și secretori lungi, uniserial; the secretory ones having hte pland pluricellular on levels;
  - the crust is defferentiated between a collencyme ring and an internal parenchymatic area;

- the central cylinder is formed of numerous libero-wooden conducting fascicles of the colateral-open type, surrounded by a thick gurdle of sclerenchym at the margins of the liber.

- at the level of the leaf:
  - the limb is hypostomatic;
  - the tector hairs are also very numerous on the inferior face of the limb and very rare on the superior one;
  - in the cross section, the median vervure is very prominent on the inferior face of the limb and less on the superior one;
  - the limb has bifacial ecvifacial structure.

Fahn (1988) said that:

- the secretory hairs of many composites are pluricllular and biserial; these are either pedicelled, or sesilled;
- the cells on their top secrete terpens, lipides, flavonoidic aglicons and wax (lipofilic material);
- in case of *Inula viscosa*, the secretory hairs produce terpens, lipids, polysaccharides, proteins;
- the substances secreted are accumulated under the cuticule, and after the coticule is torn they are eliminated to the exterior.

Nikolakaki and Christodoulakis (2004) made a study on the secretory structure in the *Inula viscosa* leaf, underlining the following peculiariries:

- the limb is amfistomatic; the stomata being of the anomocytic type;
- the presence of long tector hairs and numerous secretory hairs;
- the secretory hairs are short, pluricellular, formed of pedicell and head with two cells.

After analyzing the **structure of the vegetative organs of the studied species,** we noticed the following aspects:

1. ***The root*** **(drawing III).** At the level of the **rhizoderm** one may notice adsorbant hairs of variable length, with very thin walls.

The cortical parenchyma presents a different number of cell strata: 6-7 (*Inula conyza*), 10 (*Inula oculus-christi*), 10-12 (*Inula britannica* and *Inula germanica*) and 16-18 (*Inula ensifolia*). In case of *I. germanica*, here and there, in the thickness of the internal cortical parenchyma one may notice secretory canals, localized near the liberien fascicles. In

the internal stratum of the cortical parenchyma there are **cells with helenine** (*I. hirta, I. helenium, I. conyza*) or cells with tanin (*I. britannica*).

Helenine is a mixture of sesquiterpenic lactons, used in lung diseases, and externally as lotions against ulcer and arthritis (Grigorescu et al., 2001). In the *I. britannica* and *I. Helenium* species, in the cortical parenchyma, between the cells there are aerial canals gaps. With the majority of the studied species, the cortical parenchyma ends with an endoderm of a special type Caspary thickenings in the radial walls.

The presence of the aerial lacunary canals in the roots of *I. britannica* and *I. helenium* represents an adaptation of the plant to the environmental conditions, were the phreatic water is close to the soil surface, proving the mezohygrophile or optionally halophile character (*Inula britannica*) mentioned by Ciocarlan (2000).

The stel is of several typesi: triarh (*I. britannica, I. conyza*), tetrarh (*I. oculus-christi, I. helenium*), pentarh (*I. hirta*), hexarh (*I. salicina* ssp. *salicina, I. ensifolia*) and septarh *(Inula salicina* ssp. *aspera, I. germanica*).

The speies studied had a primary structure of the root; only *I. hirta, I. britannica* and *I. conyza* show a secondary structure at the level of the central cylinder, based on the cambium.

2. ***The rhizome*** **(drawing IV).** The epidermic celles that are isodiametric or slightly tangently prolongued, with the outer wall thicker than the others.

The crust is relatively thick,cellulose-parenchymatic, of the meatic type, formed of a variable number of cell strata: 7-8 (*I. ensifolia*), 8-10 (*I. oculus-christi*), 12-14 (*I. germanica*), 12-16 (*I. salicina* ssp. *salicina*), 16-18 (*I. salicina* ssp. *aspera*).

In *I. oculus-christi, I. salicina* ssp. *salicina, I. salicina* ssp. *aspera, I. ensifolia, I. hirta, I. germanica* and *I. britannica*, in the interior strata of the crust there are bir **secretory canals**, tangently prolongued (*I. germanica, I. britannica*), with the colector canal surrounded by a stratum of epitelial cells. These canals are more often localizedbetween the girdles of priliberien sclerenchematic fibres. With *I. Helenium*, in the thick cortical parenchyma, in the thick ring of secondare phloem abd the very thick xylem ring there dominate the **secretory canals**, as Terpilo (1961) noticed.

The conducting tisseus from the central cylinder of the rhizome have secondary structure in the majority of the species. The phellogen differentiates in *I. ensifolia* and *I. helenium*, generating suber to the exterior and phellodem to the interior.

3. ***The stem*** **(drawing V). The epidermis** has isodiametri ca nd isomorphous cells with the external and internal walls thicker than the others; the external wall is covered by a thin curicule. In some species *(I. oculus-christi, I. germanica, I. hirta, I. bifrons, I.*

conyza) one may notice, at the level of the epidermis, the presence of pluricellular tector hairs and of biserial secretory hairs.

In *Inula hirta*, *Inula salicina* ssp. *salicina* and *Inula ensifolia* we see some conducting fascisles in the crust, completely surrounded by sclerenchematic fibres.

The central cylinder is rather thick, with agreat number of libero-wooden conducting fascicles, separated by medular rays of different width, having cells with the walls moderately thickened and lignified at the wood level. The conducting fascicles present, at the margins of the liber, each a very thick girdle of sclerenchematic fibres, with thickened and intensly lignified walls.

The pith is either cellulose-parenchymatic, or parenchymatic-lignified, the cells having the walls moderately thickened.

Towards the basis, the general structure remains the same, with the difference that the cross section contour becomes almost circular, the wood quantity is greater, the lignifications is more intense and the pith thicker, some of the cells of the last get disorganized, resulting in some aerial cavities with irregular margins. At the basis of the stem, the number of the hairs is reduced, the number of the mechanical cortical fibres increases, as well as the thickness of the periliberien mechanical girdles.

4. ***The leaf*** **(drawings VI, VII)**. **The petiole** present at the basal leaves of *I. oculus-christi*, *I. helenium* and *I. conyza* in the cross section is realtively cordiform (*I. oculus-christi*) or approximately circular (*I. helenium*, *I. conyza*), with lateral-abaxial ribs that separate a superficial adaxial ditch (*I. oculus-christi*). On the abaxial face of the petiole, the tector hairs are numerous, thick, pluricellular, with very long terminal cells, and the secretory hairs are pluricellular, biserial (*I. helenium* and *I. conyza)*. In *I. oculus-christi*, the tector and the secretory hairs are uniformly distributed both on the adaxial face and of the abaxial one. In the fundamental parenchyma, the conducting tissues are represented by the libero-wooden fascicles, disposed on an arch.

The limb is amfistomatic (*I. salicina ssp. salicina, I. ensifolia, I. hirta, I. britannica, I. conyza*) or hypostomatic (*I. salicina ssp.aspera, I. germanica*). The limb structure is heterofacial bifacial (dorsiventral),with unistratified palisadic tissue (*I. salicina ssp. salicina, I. salicina ssp.aspera, I. helenium, I. bifrons, I. britannica*) or bistratified (*I. germanica*). In *I. ensifolia, I. hirta, I. conyza*, the limb presents an isofacial bifacial structure (the mesophyl is homogenous, relatively compact, formed of rounded cells, only with meates between them).

As aresult of the histo-anatomical analysis of the vegetative organs, we highlight several differences an similarities between the two subspecies of *I. salicina*: ssp. *salicina* and ssp. *aspera* .

Thus, at root level, *I. salicina* ssp. *Salicina* presents 6 wooden liber fascicles while in *I. salicina* ssp. *aspera,* there are 7-8 wooden conducting fascicles, that alternate with as many liber fascicles. In both subspecies, in the rhizome, in the internal stratum of the internal cortical parenchyma there are secretory canals.

The contour of the superior third cross section of the aerial stem is circular ribbed, with 8 relatively proeminent ribs and relatively deep valecules in case of the *I. salicina* ssp. *salicina* subspecies and circular, unregularly ribbed, with different size ribs in case of the *I. salicina* ssp. *aspera* subspecies.

At the level of the inferior aerial stem third, *I. salicina* ssp. *aspera* has thich, uniserial tector hairs, and also visibly proeminent stoamata over the protecting tissue level.

In the *I. salicina* ssp. *Salicina* subspecies, the limb is amfistomatic, while in *I. salicina* ssp. *aspera*, the stomata are missing from the suoerior epidermis (so the limb is hypostomatic). In *Inula salicina* ssp. *aspera*, near the nervures of the foliar limb there are long, uniserial tector hairs.

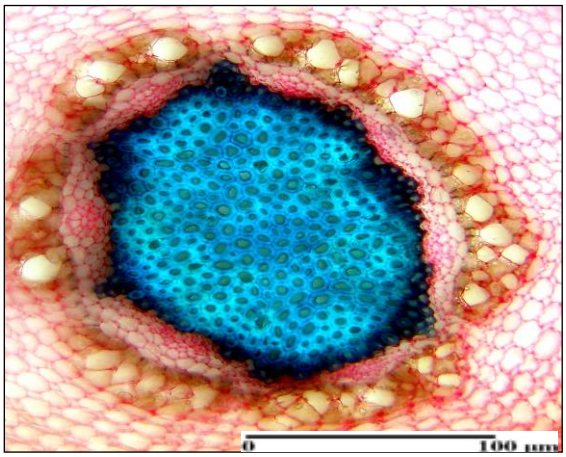

fig.14

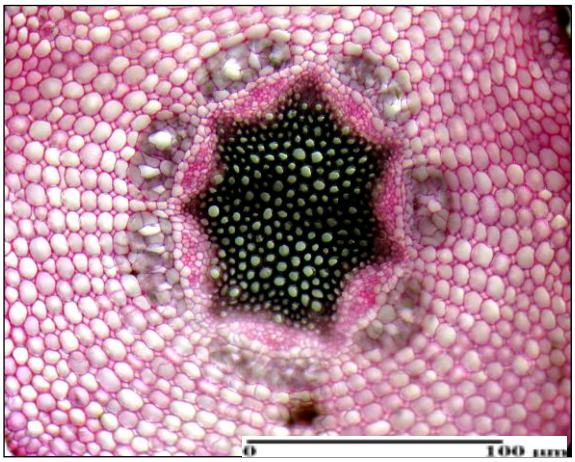

fig.15

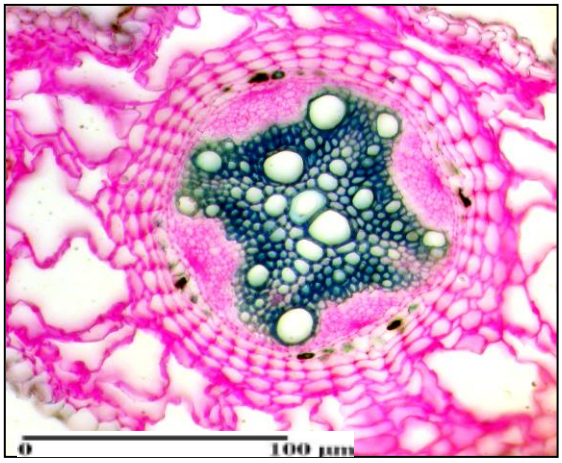

fig.16

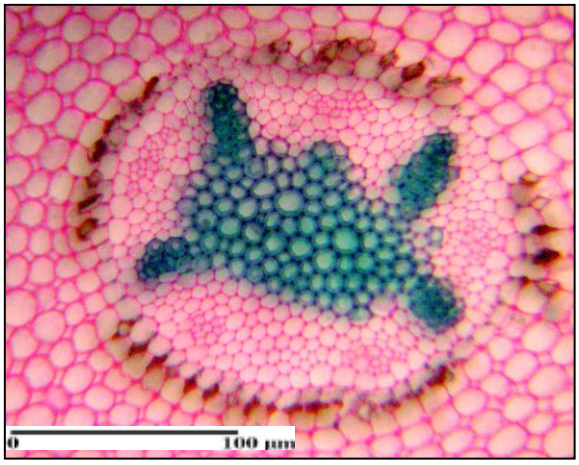

fig.17

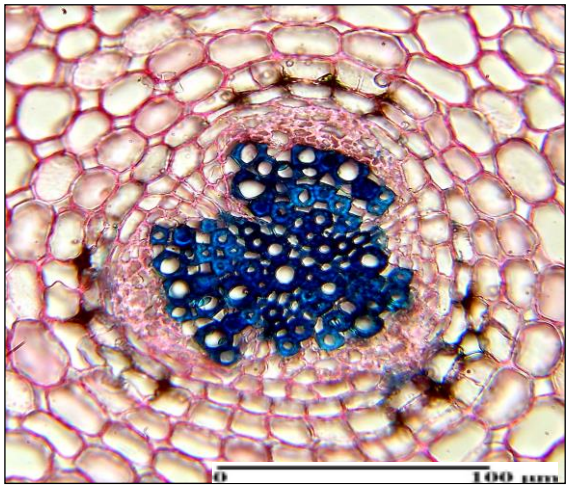

fig.18

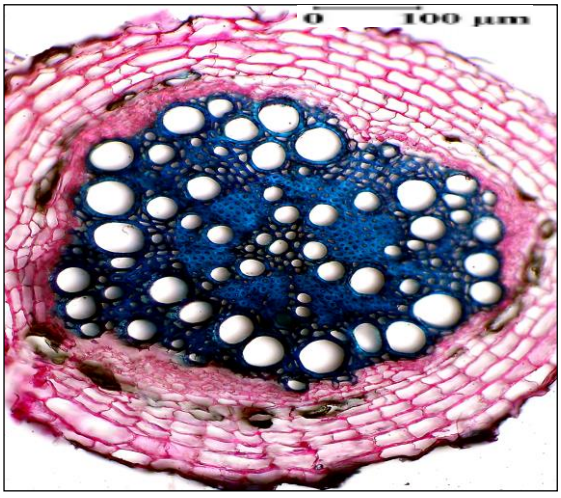

fig.19

Drawing III. Root cross sections – central cylinder detail: *I. salicina* ssp. *salicina* (fig. 14), *I. germanica* (fig. 15), *I. helenium* (fig. 16), *I. ensifolia* (fig. 17), *I. britannica* (fig. 18), *I. conyza* (fig. 19) (orig.)

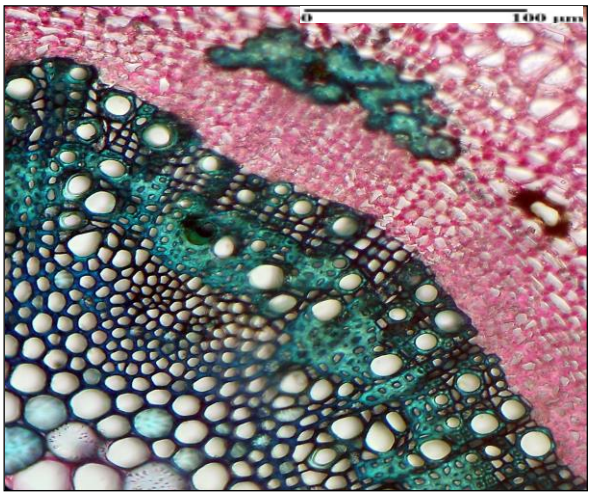

fig.20

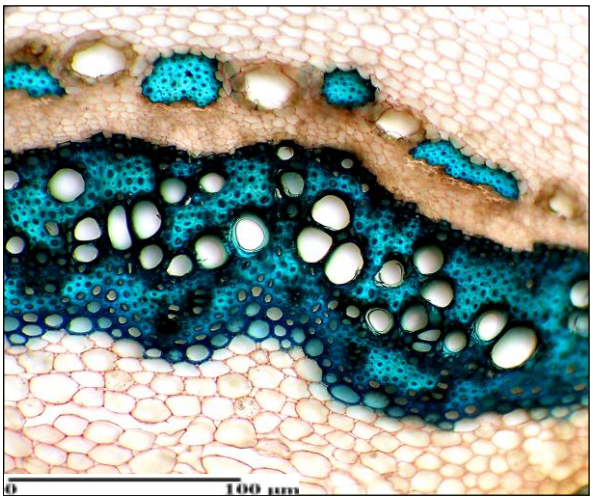

fig.21

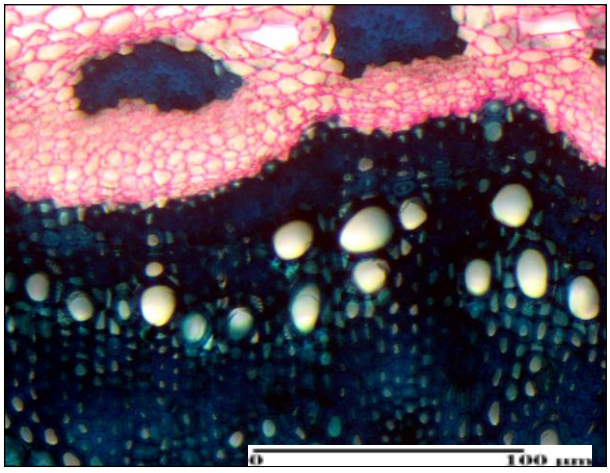

fig.22

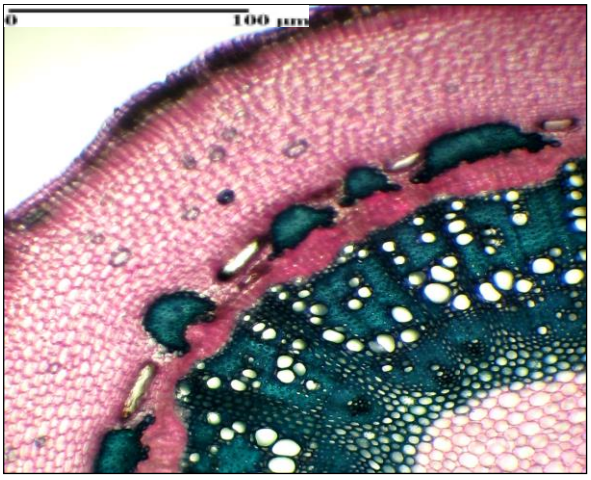

fig.23

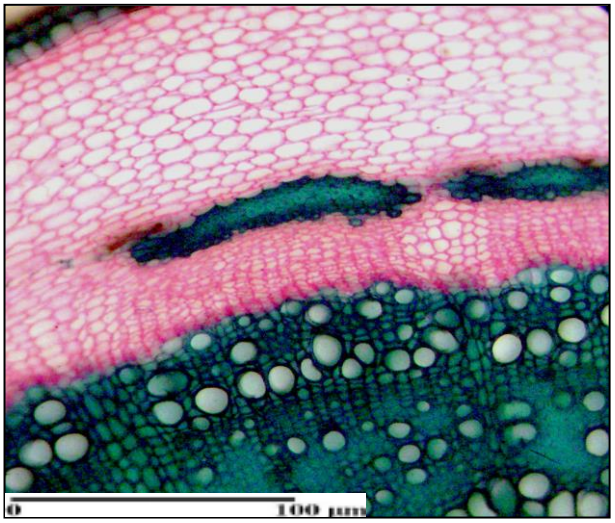

fig.24

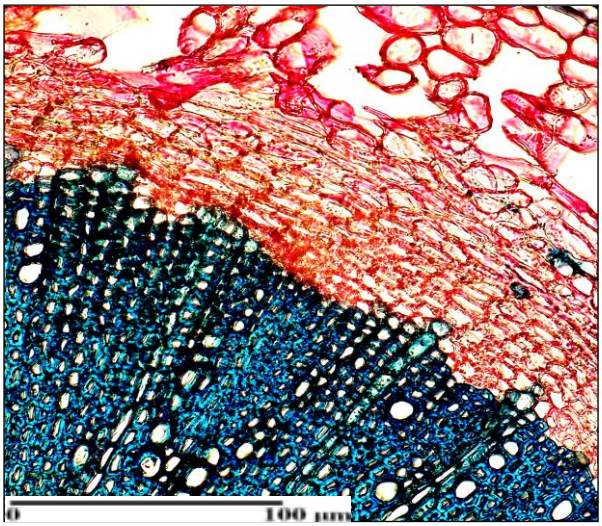

fig.25

Drawing IV. Rhizome cross section *I. oculus-christi* (fig. 20), *I. salicina* ssp. *salicina* (fig. 21), *I. salicina* ssp. *aspera* (fig. 22), *I. germanica* (fig. 23), *I. ensifolia* (fig. 24), *I. britannica* (fig. 25) (orig.)

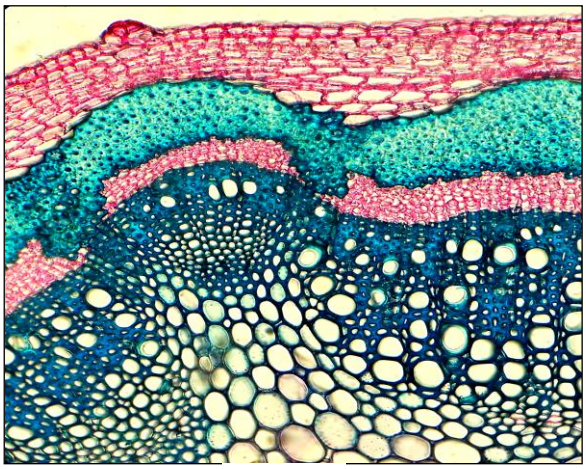

fig.26

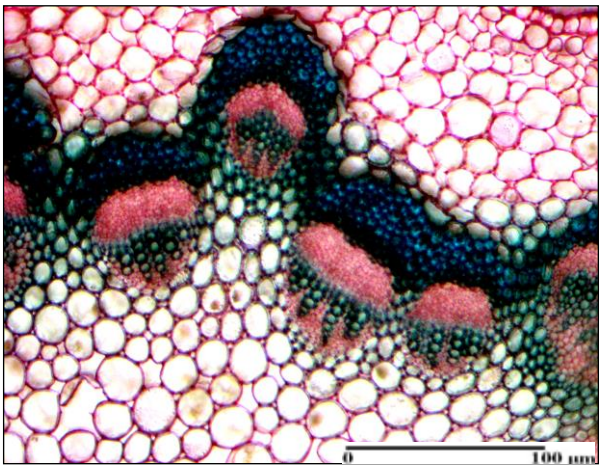

fig.27

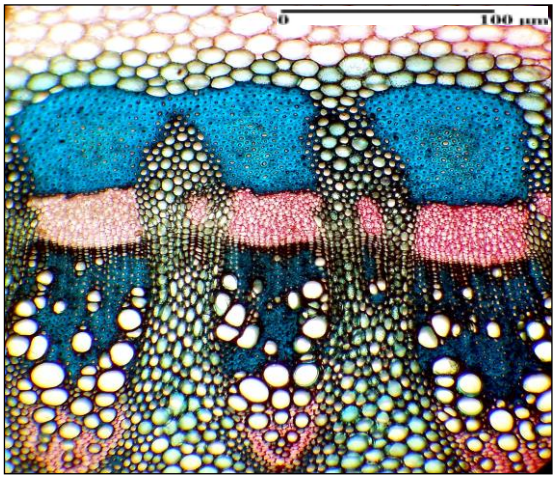

fig.28

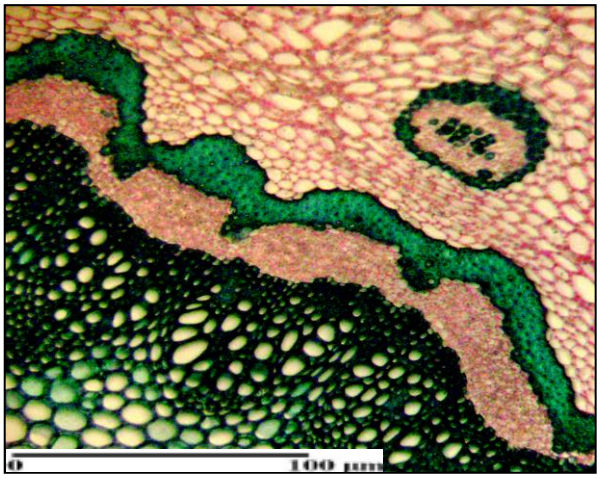

fig.29

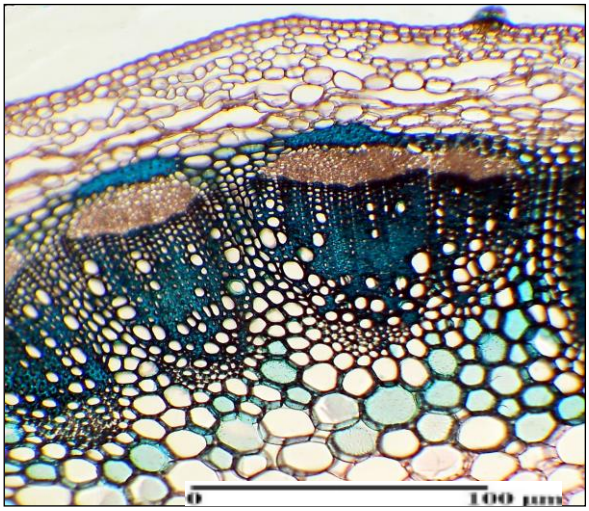

fig.30

fig.31

Drawing V. Aerial stem cross section: *I. salicina* ssp. *salicina* (fig. 26), *I. salicina* ssp. *aspera* (fig. 27), *I. helenium* (fig. 28), *I. hirta* (fig. 29), *I. britannica* (fig. 30), *I. bifrons* (fig. 31) (orig.)

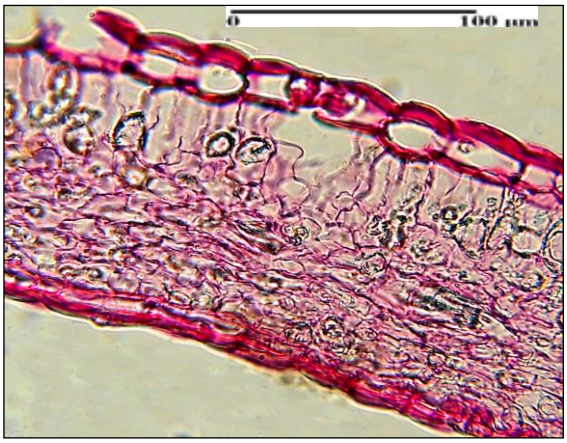

fig.32

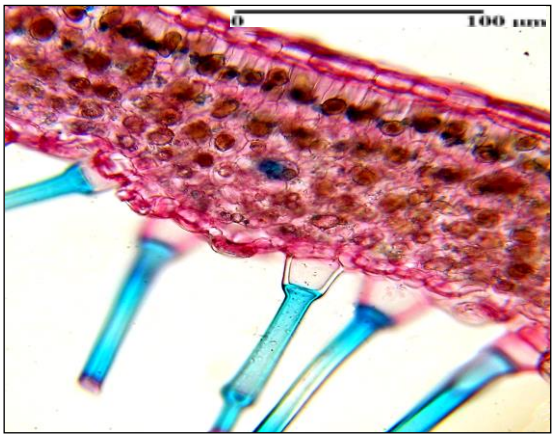

fig.33

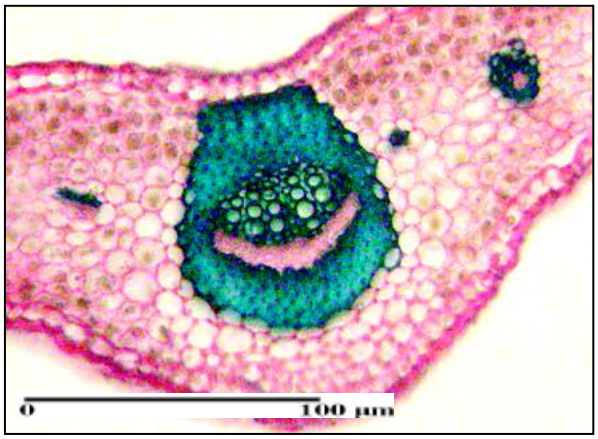

fig.34

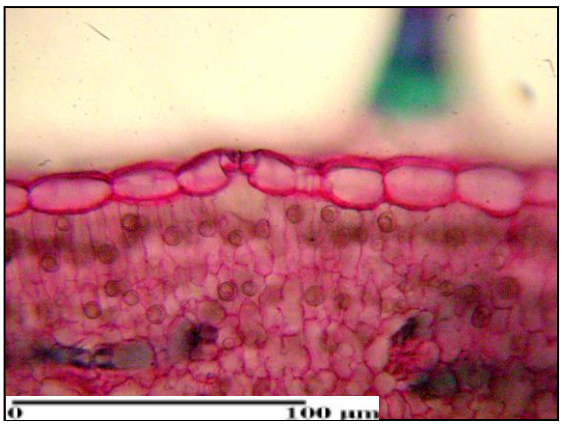

fig.35

fig.36

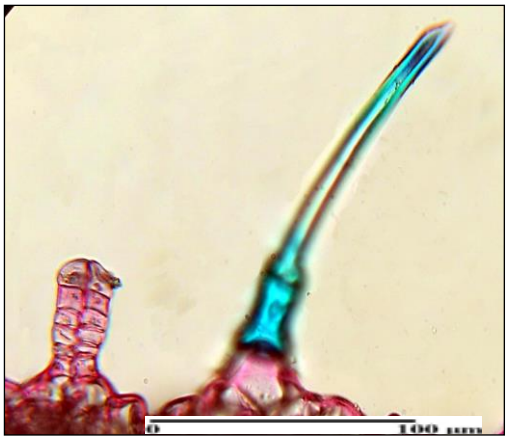

fig.37

Drawing VI. Leaf cross section: *I. salicina* ssp. *salicina* (fig. 32), *I. helenium* (fig. 33), *I. ensifolia* (fig. 34), *I. hirta* (fig. 35), *I. britannica* (fig. 36); *I. bifrons*: tector and secretory hairs (fig. 37) (orig.)

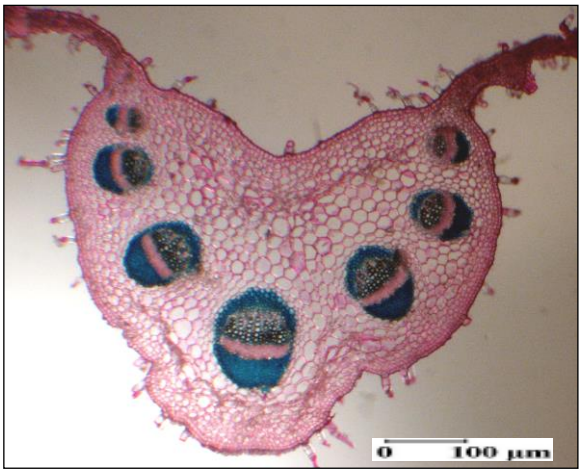

fig.38

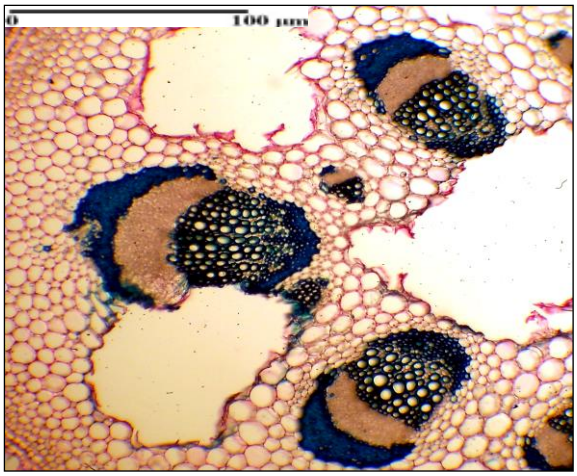

fig.39

fig.40

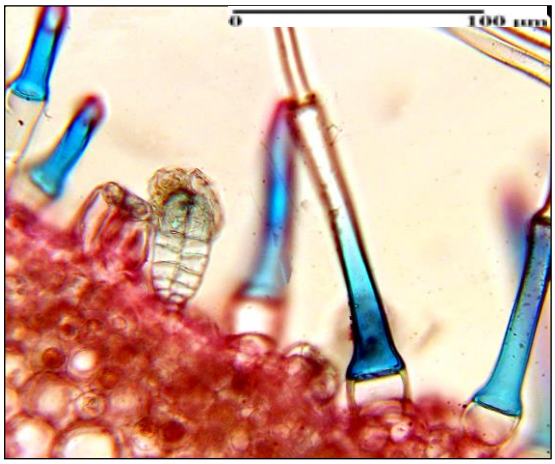

fig.41

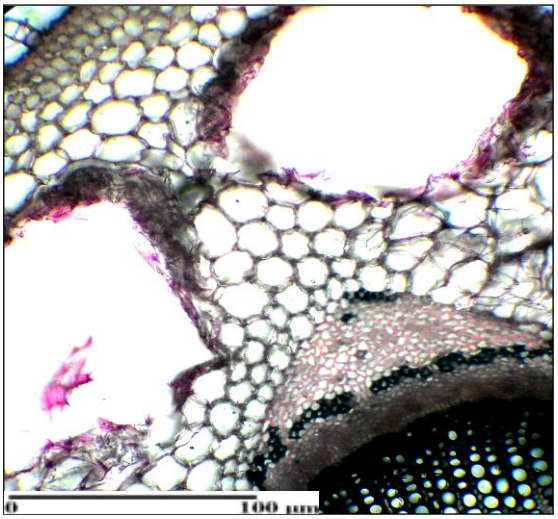

fig.42

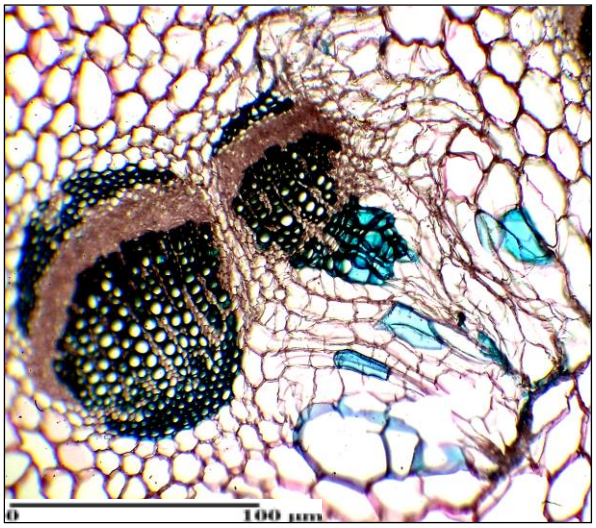

fig.43

Drawing VII. Petiole cross section: general view *I. oculus-christi* (fig. 38); detail: conducting fascicle, aerial cavities, tector and secretory hairs. *helenium* (fig. 39-41); detail: aerial cavities and conducting fascicles *I. conyza* (fig. 42, 43) (orig.)

❑ Morphological aspects of the Inula L. species

• Morphological aspects of the *Inula* L. leaf (drawing VIII)

As aresult of the foliar surface of the *Inula* species, with the help of the electronic microscope with scavenging, we highlighted the following:

- the external surface epidermic cell walls has a different conformation: ondulated in the *I. ensifolia*, *I. oculus-christi* and *I. Germanica* species, with cuticular crests at *I. britannica* or with obvious scratches at *I. salicina* ssp. *aspera*;
- the stomata are present in th esuperior epidermis of *I. oculus-christi, I. ensifolia*, *I. salicina* ssp. *salicina*, *I. britannica*, *I. hirta, I. conyza*. These are missing from the superior epidermis of the *Inula salicina* ssp. *Aspera species*;
- the stomata are surrounded by annex cells with the exterior surface modified, presenting either lamellar excrescences of epicuticullar wax (la *I. ensifolia*), or radially disposed ondulations (*I. salicina* ssp. *salicina*) or proeminent crests that limit each stomatic apparatus (*I. britannica*);
- in most of the cases, the opening of the stomata is surrounded by a waxless cuticullar collaret (*I. germanica*) or by moderate ondulations of the cuticule, disposed in parallel to the stomata axis (*I. britannica*);
- at the level of the superior epidermis one may notice two types of hairs: uniserial plirucellular tector hairs, (the majority with 1, 2, 3 and 4 short basal cells and and one or two long and very long terminal cells) and secretory hairs with clavated gland;
- the external surface of the cells from the inferior epidermis presents ondulations (*I. oculus-christi*), parallel cuticullar scratches (*I. ensifolia*, *I. salicina* ssp. *aspera*) or frequent cuticullar crests (*I. britannica*);
- in case of all the analyzed speceis, the inferior epidermis there are stomata. The annex cell walls of the stomata present ont eir surface wax lamellas either aranged, isolated, non-stratified (*I. ensifolia*), or orderly disposed, with parallel lamellas (*I. salicina* ssp. *aspera*). In *I. hirta* and *I. Germanica* species, the opening of the stomata is surrounded a waxed collaret;
- at the level of the inferior epidermis we observe two types of hairs: pluricellular tector hairs, long, numerous, mostly disposed on the nervures (*I. oculus-christi*, *I. hirta*, *I. salicina* ssp. *aspera*, *I. helenium*, *I. conyza*, *I. britannica*) and secretory hairs, a greater number in the *I. Helenium* species.

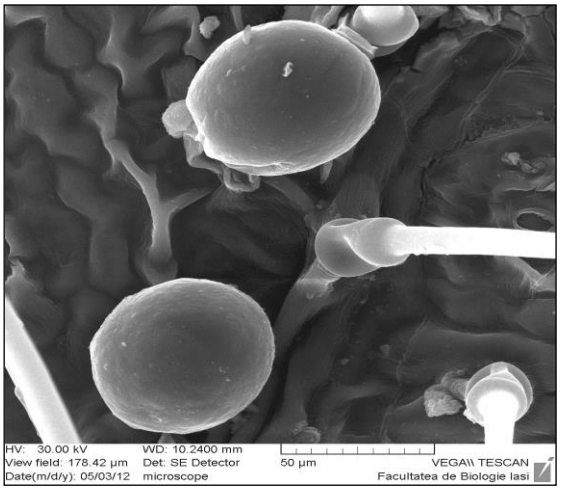

fig.44

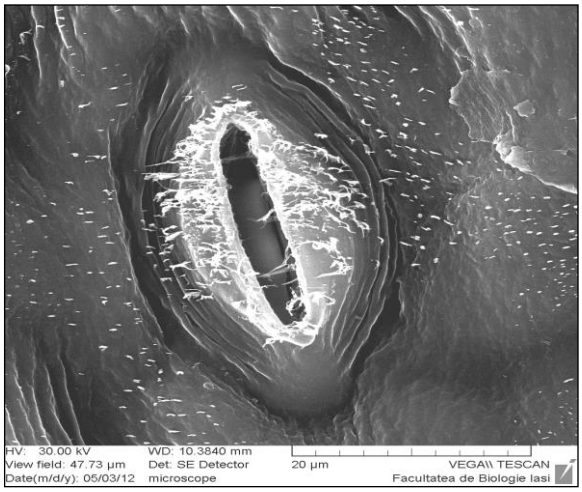

fig.45

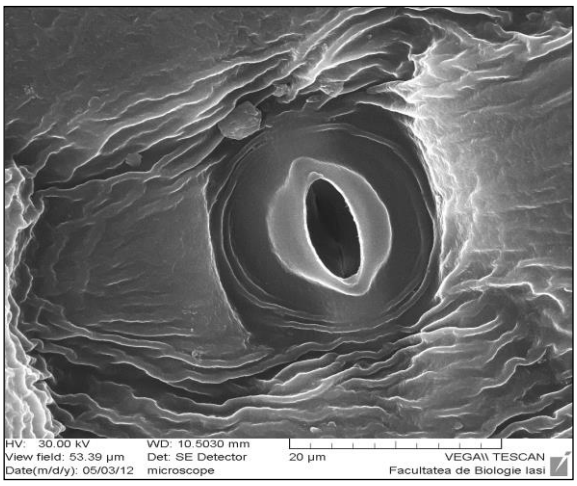

fig.46

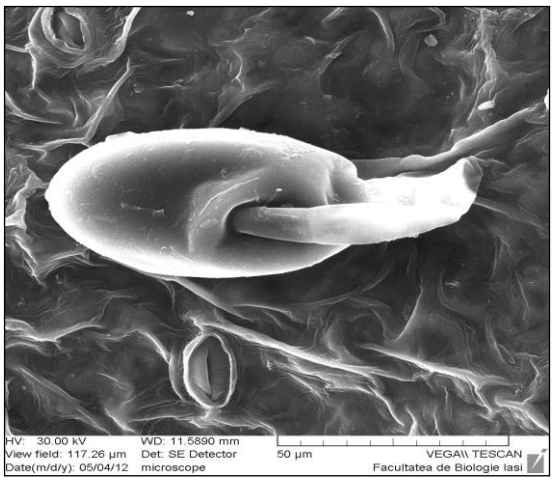

fig.47

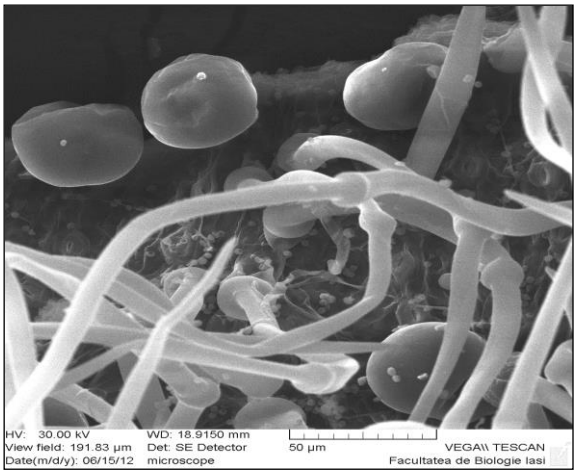

fig.48

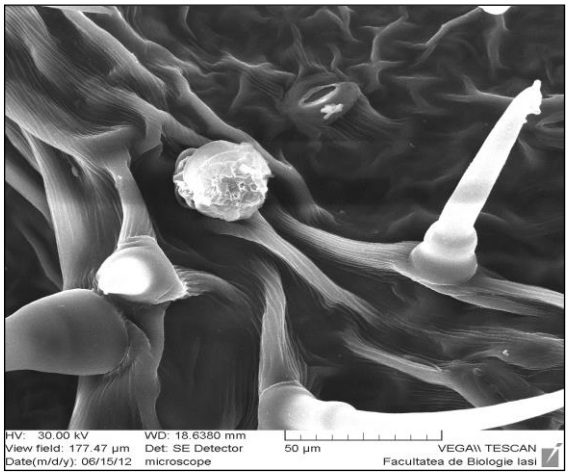

fig.49

Drawing VIII. *I. oculus-christi*: secretory and tector hairs (fig. 44); *I. ensifolia*: detail: inferior epidermic stomatum (fig. 45); *I. salicina* ssp. *salicina*: detail: superior epidermic stomatum (fig. 46); *I. britannica*: detail: inferior epidermic secretary hair (fig. 47); *I. helenium*: secretory and tector hairs of the inferior epidermis (fig. 48); *I. conyza*: tector and secretory hair of the inferior epidermis fig. 49) (orig.)

Date regarding the morphology of the fruits of the *Inula* species (fig. 50-53)

Boyko (2011) studies by means of the electronic microscope the surface of the different *Asteraceae* species achenes. On the basis of the results, there have been identified diverse morphological types of hairs. The authore do not characterize the species of the *Inula* genus.

The surface of the pericarp in the speccies of ***Inula*** **studied by us** is ribbed. The pericarp presents narrow polygonal cells, with an extremely smooth exterior wall (*I. helenium*, *I. hirta)* or alongated (*I. germanica*, *I. conyza*), sometimes with sharp tips (*I. hirta*), many times covered by a thicker cuticullar complex, slightly goffered (*I. ensifolia*) or with an amorphous aspect (*I. germanica*, *I. conyza*). On the surface of the pericarp there are relatively short tector hairs, with a sharp tip (*I. ensifolia*), with short, slightly maimed hairs (*I. bifrons*) or robust alongated ones (*I. conyza*).

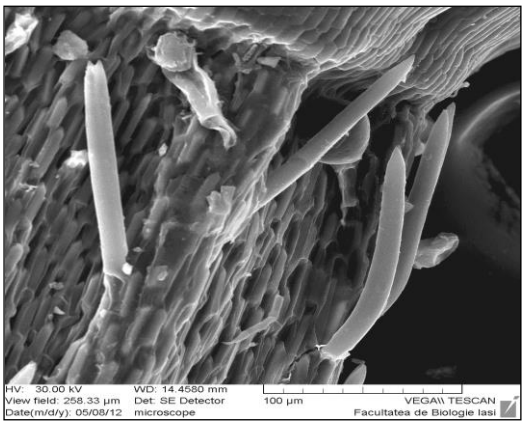

fig.50

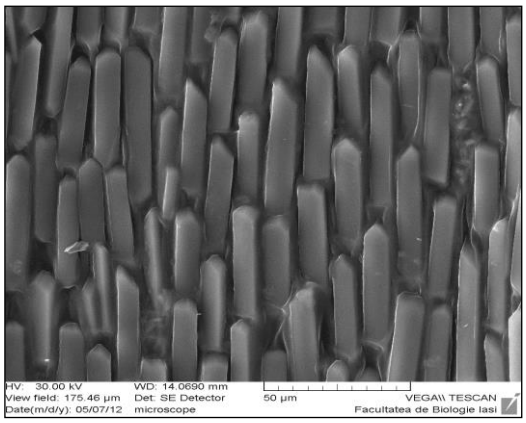

fig.51

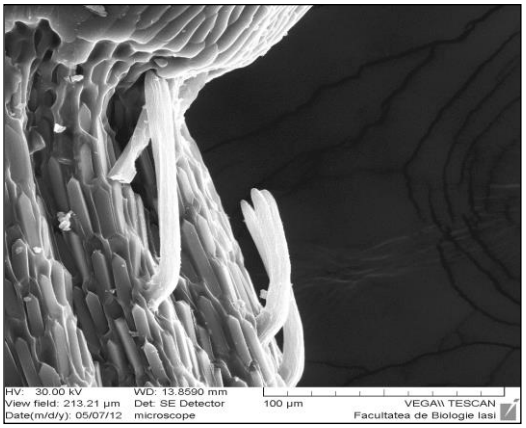

fig.52

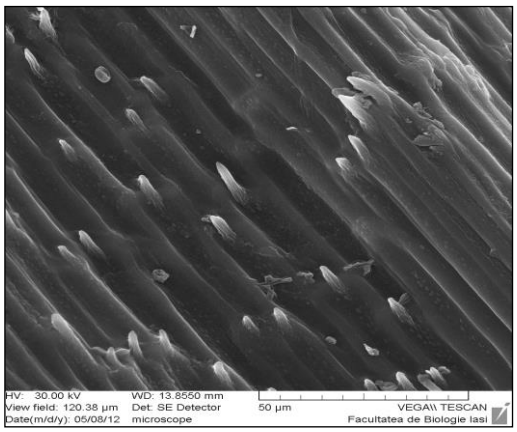

fig.53

Inula ensifolia- achene: detail: pericarp with tector hairs (fig. 50); *Inula hirta*- achene, detail: pericarp cells (fig. 51); *Inula conyza*: detail: tector hairs onthe surface of the pericarp (fig. 52); *Inula bifrons*, detail: tector hairs on the pericarp surface (fig. 53) (orig.)

- **The characterization of the mitotic chromosomes of the studied Inula L. species**

- **The morphological characteristics of the *Inula spiraefolia* L. chromosomes**

The analysis of the well exposed chromosomes, with less than two superpositions, allowed us to establish thediploid number to be equal with 16, so that 2n=16, in concordance with th data of scientific literature and the cariotype achieving (fig.54 and 55).

Taking into account the ratio among the branches (B_L/B_S), the centromeric index, the relative length and the branch differences (B_L-B_S), we could establish the fact that the *Inula spiraefolia* L. presents a single type of chromosomes: median (m), all the eight pairs of homologues (I – VIII) the relation between the branches from 1.09 and 1.49.

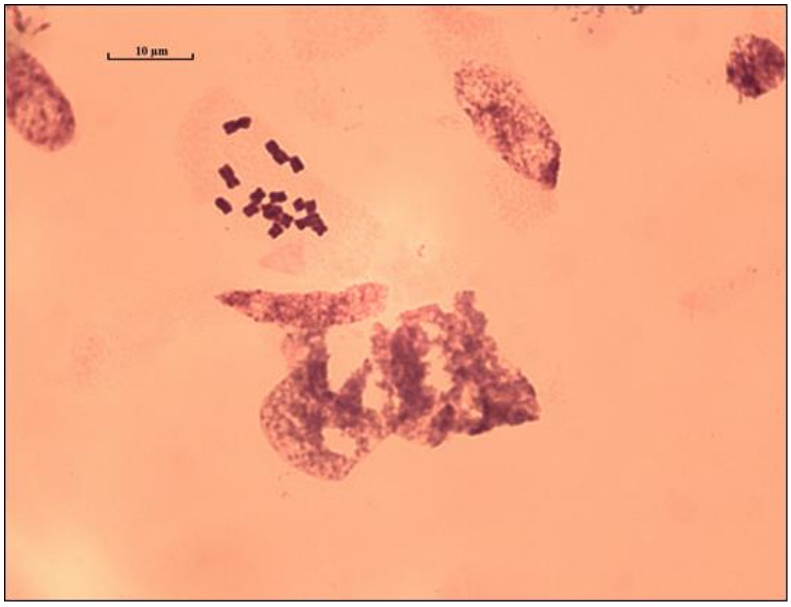

fig.54. Metaphase – *Inula spiraeifolia* L. (2n=16)

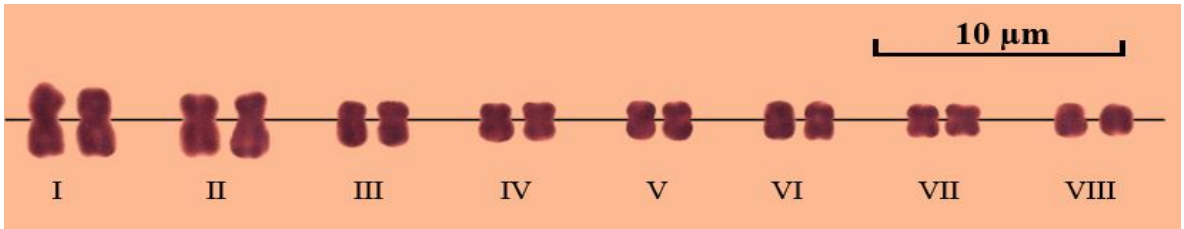

fig.55. Cariotype *Inula spiraeifolia* L. (2n=16)

- **The morphological characterisitics of the *Inula ensifolia* L. chromosomes**

In the works we consulted we found that with the *Inula ensifolia* L. species, the number of the chromosomes in the somatic cells is 2n = 16, all the samples studied being diploid. In the speciality literature, we found a single work (Kamari et al., 2008) describing the morphology and typolgy of this species chromocomes, yet in case of one triploid variant $2n = 24 = 3x = 16m + 4m^{SAT} + 2sm^{SAT} + 2t^{SAT}$ our study has the character of novelty.

Taking into account the relation among he branches (B_L/B_S), the centromeric index, the relative length and the difference of the branches (B_L-B_S), we coulg establish the fact that the *Inula ensifolia* L. species presents two types of chromosmes median (m): the pairs I – VII (having th erelation between the branches between 1.03 and 1.53) and submedians (sm): pair VIII, having the ratio between the branches of 1,.7 (fig.57).

fig.56. Metaphase – *Inula ensifolia* L. (2n=16)

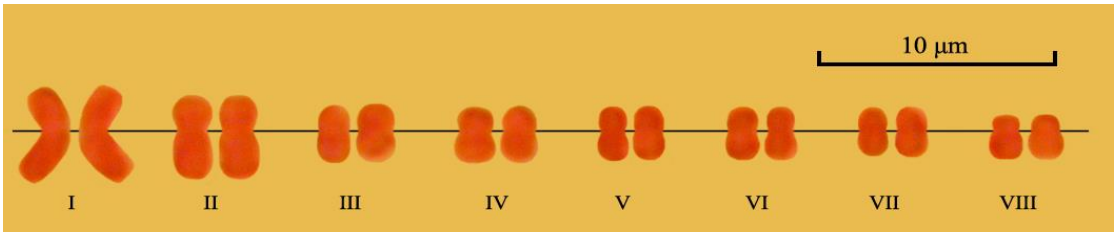

fig.57.Cariotype - *Inula ensifolia* L. (2n=16)

- **The morphological characteristics of the *Inula hirta* L. species chromosomes**

In case of the *Inula hirta* L. species, we tried to characterize the chromosome from the morphometrical and typological point of view due to the stages stated in chapter II; unfortunately, this objective could not totally be finalized: becuse of the different degree of spiral winding of some homologues we obtained only two variants of cariotype.

Taking into account the relation among the branches (B_L/B_S), the centromeric index, the relative length and the difference of the branches (B_L-B_S), we could establish the fact that that the *Inula hirta* L. species presints two types of chromosomes: median (m), the pairs of homologues I, II, III, IV, VII și VIII (having th ratio between tyhe branches from 1.15 and 1.50) and the **submedians**, the homologues pairs **V** and **VI** (having the ratio between the branches from 2.13 and 2.54) (variant 1; fig. 59).

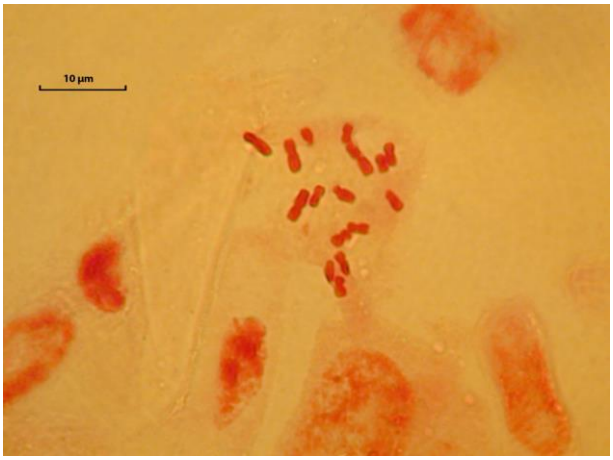

fig.58. Metaphase 1 – *Inula hirta* L. (2n=16)

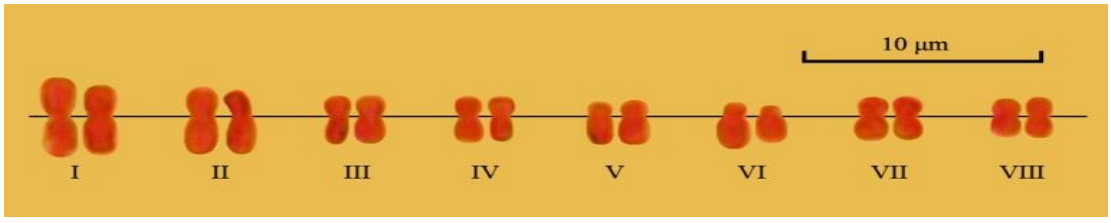

fig.59. Cariotype 1 - *Inula hirta* L. (2n=16)

In case of metaphase 2, taking into account the ratio between the branches (B_L/B_S), the centromeric index, the relative length and the branches difference (B_L-B_S), we could establish on this metaphase too that the *Inula hirta* L. presents two types of median chromosomes: median (m), the homologues pairs I, II, IV, V, VI and VII (having the ratio between the branches from 1.05 to 1.64) and **submedians**, the homologues pairs **III** and **VIII** (having the ratio between the branches from 1.82 and 2.27) (fig 61).

fig.60. Metaphase 2 – *Inula hirta* L. (2n=16)

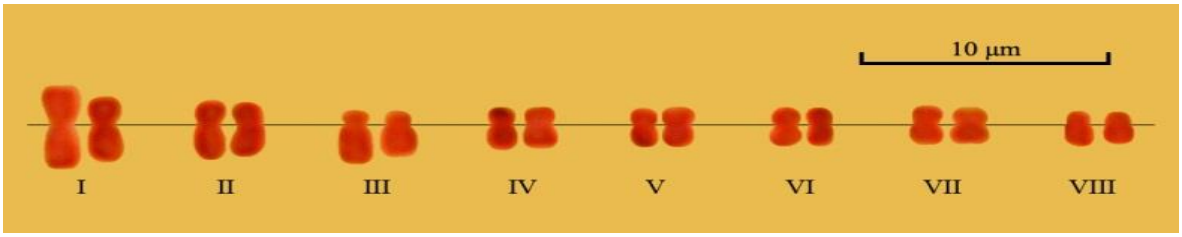

fig.61. Cariotype 2 - *Inula hirta* L. (2n=16)

- **The morphological characterization of the *Inula helenium* L. species chromosomes**

The analysis of the well exposed chromosomes, but in metaphases with two or more superpositions, allowed us to confirm that the diploid number as being equal to 20, thus 2n=20, concording with data of speciality literature (Kokubugata et al., 2002), and the achieving of the cariotype.

Without performing all the measurements, we may notice from the analyzed metaphases two categories of chromosomes: medians (m) and submedians (sm); we appreciate that the typology of the chromosomes is closer to the researches made by Ruolin and Wenbing in 1985, than those of Kokubugata et al., in 2002.

Using the classical cytogenetic techniques, we succeeded to identify secondary constrictions that represent satilferous peduncles and satelites on a pair of submetacentric chromosomes (fig.62), thus in conformity with the data from the speciality literature (Ruolin and Wenbing, 1985 and Kokubugata et al., 2002).

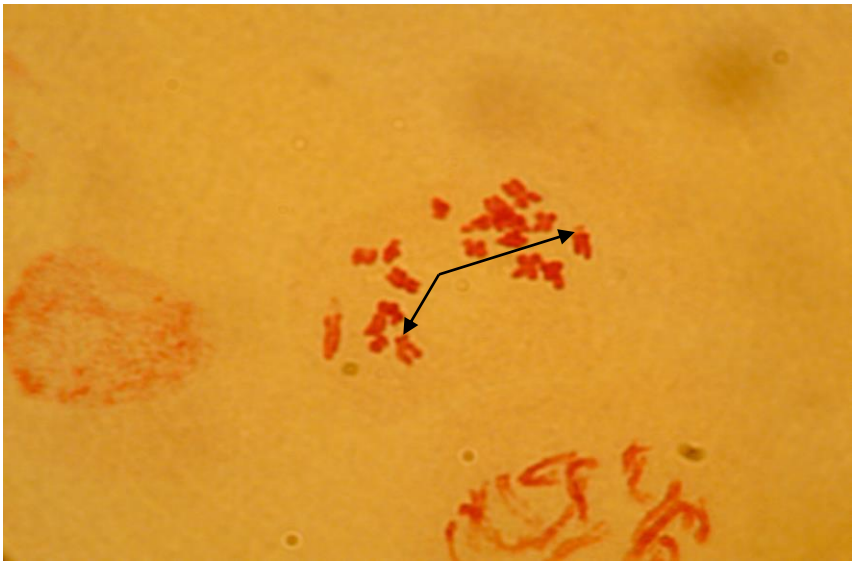

fig.62. Metaphase of the *Inula helenium* L. species (2n=20) – the satelites are marked on the short branches of a pair of sm chromosomes

❑ The qualitative and quantitative determination of the active principles of L. (elecampane)

- **The qualitative analysis**

The qualitative analyssis by thin layer chromatography (TLC) highlighted the presence of volatile, terpenic, flavonoids and polyphenolic compounds.

- in the analized volatile oil we identified: limonen, linalol and β-pinen;
- the triterpenic compounds are represented by stigmasterol and oleanolic acid;
- the identified polyphenolcharboxylic acids are due to the standards: caffeic, chlorogenic, p-cumaric and ferulic acids.

- **Quantitative analysis**

The quantitative phytochemical analysis demonstrated the existence of fructose (inuline) in great quantities, the average per experiment being of 49.10 g% d.w, of rutosid and caffeic acid, and of the total aminoacids expressed in glutamic acid.

Conclusions

❖ The results regarding the histo-anatomic of the vegetative organs of the *Inula* species from the Romanian flora confirmed, on the one hand the already known data (special reference to *I. helenium*), and on the other hand completed the data of the speciality literature, known being the fact that there are few articles referring to the antomy of these species.

❖ **From the anatomical point of view**:

- in case of the analyzed plantlets and mature plants, at the level of the leaf we identified two types: massive and thick **tector hairs**, that differ in size, some being bicellular, others pluricellular, with great basal cells, sometimes almost izodiametric, and **secretory hairs** with a long pedicell, formed of two lines of cells (biserial) with bicellular gland, in a great number in *I. helenium*.

- at the root level of the mature plants, in the internal stratum of the cortical parenchyma, in the majority of the analyzed species, there are **cells with helenine**, excepting *I. germanica*, which presents in the thickness of the interior cortical parenchyma **secretory canals. The secretory canals** are present especially in the **rhizome**; the secretory canal disposition differs, inmost of the cases are localized in the internal strarta of the crust, among the girdles of sclerenchimatic periliberien fibers.

❖ **From the cytogenetic point of view:**

- we achieved the cariotype and the idiogram of 3 *Inula*: *Inula spiraeifolia*, *Inula ensifolia* and *Inula hirta* species, all with 2n=16. The 3 species differ by the morphological types of the chromosomes ; in *I. spiraeifolia*, all the chromosomes are of the median type (**m**); in *I. ensifolia*, the cariotype is represented by the 7 pairs of the median type (**m**) and a pair of submedian chromosomes (**sm**); and *I. hirta*, as wel as in the precedent species we identified 2 types of chromosomes: median and **submedian** (variant 1: the pairs V and VI; variant 2: the pairs III and VIII). With *I. helenium*, we could establish only the number of diploid chromosomes, 2n=20, and from the photos obtained we could distinguish chromosomes with satelites.

❖ After the **phytochemical studiy** achieved on samples of ***Inula helenium***, the Miclauseni-Iasi we noticed: the presence of the volatile compounds (limonen, linalol and β-pinen), terpenic compounds (stigmasterol and oleanolic acid), flavonoidic compounds (cvercetol and rutosid, also being present some of their aglicons) and polyphenolic compoungs (caffeic, chlorogenic, p-cumaric and ferulic acids) in vegetal extracts of *Inula helenium* and it was demonstrated the existence of fruictosans (inuline) in great quantities, the average per experiment beingd of 49.10 g% d.w, of rutosid and caffeic acid, and also of he total aminoacids expressed in glutamic acid.

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