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THE INFLUENCE OF SOME FERTILIZERS AND BIOSTIMULANTS UPON THE STEM ANATOMY OF *CHRYSANTHEMUM INDICUM* L. (Ist NOTE)

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Abstract. The results presented in this paper belong to the project "Elaborarea de soluţii şi tehnici de cultură neconvenţionale şi nepoluante la plantele ornamentale, în contextul dezvoltării durabile – The elaboration of unconventional and unpollutant solutions and culture techniques, in stable usage context" and is focused on the identification of structural modifications of *Chrysanthemum indicum* L. stem, as a consequence of treating plants with 3 types of foliar fertilizers and biostimulants (Maxiroot, Dacmarinur Maxi N, Aurora) in 3 variants of concentrations (0.2%, 0.4%, 0.6%). The cross sections through the stem indicate a variable diameter, depending on the concentration and the applied product. The modifications appeared in the sclerification and lignification degree, development of the pith, cortex, conductive and mechanic tissues. The study recommends the usage of unpollutant foliar fertilizers and biostimulants based on plant extract, in order to develop the elements which increase plant resistance in sustaining the inflorescence, favoring their utilitarian (economic) aspects of maintaining "cut flowers".

Key words: anatomic modifications, stem, *Chrysanthemum indicum*, fertilizers, biostimulants

Introduction

The culture of *Chrysanthemum indicum* L. in Romania is one of the most important flower sources, from economic point of view, facilitating the enlargement and enrichment of floral assortment during the year [VIDRAŞCU & MITITIUC, 2001; VIDRAŞCU & al., 1986; VIDRAŞCU & al., 1985]. *Chrysanthemum indicum* is one of the parental species of the cultivars which are nowadays in culture. The big interest manifested by the lovers of flowers for the culture of chrysanthemums is explained by their decorative qualities and their possibilities of long term usage [VIDRAŞCU & MITITIUC, 2001; VIDRAŞCU & al., 1986]. The results presented in this paper belong to the project "Elaborarea de soluţii şi tehnici de cultură neconvenţionale şi nepoluante la plantele ornamentale, în contextul dezvoltării durabile – The elaboration of unconventional and unpollutant solutions and culture techniques, in stable usage context" and is focused on testing the action of some fertilizers and biostimulants with unpollutant properties which may upgrade the classic culture technology that determine plant growing and development, including the increment of the decorative aspect [BIREESCU & al., 2002; DORNEANU & al., 2001; GAVRILUŢĂ & al., 2005].

The literature shows that in Romania *Chrysanthemum indicum* was taken into a special anatomic study [NIŢĂ & al., 2001] that is why in the present paper our interest is focused on the anatomic modifications appeared after the treatment with some fertilizers and biostimulants, in various concentrations, with the purpose of recommending their usage in culture technologies, in order to obtain plants of higher quality. The identification of the impact made by the above products could be explained by analyzing the anatomic

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modifications appeared in the stem (first note), knowing that the resistance of the inflorescence on the plants, as well as plant resistance to fall depends on the development and thickness of the stem tissues [TOMA, 1975, 1977; TOMA & al., 1985; TOMA & GOSTIN, 2000].

Material and methods

The experimental cultures have been initiated at the University of Agricultural Sciences and Veterinary Medicine of Iasi, and have been ordered in randomized blocks, with three repetitions. Four foliar treatments have been applied, at 10 days intervals, on various variants (V_1 – 0.2%, V_2 – 0.4%, V_3 – 0.6%), using the following products: Maxiroot, Dacmarinur Maxi N and Aurora. Maxiroot is a foliar fertilizer with biostimulating effect: N – 2%, K – 4%, Zn – 0.3%, Fe – 0.3%, organic material – 30%, free amino acids (tryptophan, arginine) – 3%, proteins, vitamins. Dacmarinur Maxi N is an ecologic foliar fertilizer from marine algae (*Ascophyllum nodosum*): N – 216 g/l, K – 96 g/l, P – 38 g/l, cytochinons – 7.5 mg/l, auxines – 11 mg/l, amino acids, vitamins, proteins. Aurora is a Romanian natural extract from plants, with biostimulant effect and contains N – 10%, K – 3.5%, P – 0.5%, B, Ca, Cu, Fe, Mg, Zn – 2% each of them, enzymes, amino acids, vitamins.

The stems have been preserved in ethylic alcohol 70% and cross sectioned at middle level. The sections were coloured with iodine green and carmine red and mounted in gel. The histological cuttings were analyzed in a Novex (Holland) microscope and photographed by means of a Sony DSC-W5/W7/W15/W17 photo camera.

Results and discussions

Maxiroot 0.2% (V_1). Young stem. The shape of the cross section is circular-ribbed, with rounded ribs. Epidermis bear tangentially elongated cells, having thickened external and internal walls (Pl. I: Fig. 4); the external wall is covered by a thin cuticle. Here and there, stomata are present, situated above the epidermic cells, as well as multicellular one-layered protective hairs and secretory trichomes.

The cortex is quite thin (5-6 layers of cells); the first subepidermic layer and the layers which belong to the ribs have the walls of the cells thickened that the rest. The cortex ends in a primary endodermis with small and ordered cells.

The vascular tissues form numerous phloemic-xylemic bundles of collaterally-opened type (Pl. I: Fig. 4), separated by weak lignified and sclerified medullary rays; there is an alternation between small and big bundles. The phloem forms a thin region of sieved tubes, guard cells and a few parenchyma cells. The xylem bears vessels separated by libriform at the external part and cellulosed parenchyma at the internal part. At the periphery of the phloem there is a sheath of mechanic fibers, with wide lumina and moderately thickened walls (Pl. I: Fig. 4).

The pith is thick, parenchymatous, with a lignificated perimedullar region.

Maxiroot 0.2% (V_1). Mature stem. The shape of the cross section is circular-ribbed, with wider ribs (Pl. I: Fig. 1). The diameter of the stem is twice bigger. The structure is of fascicular type. The epidermis bears cells with thickened external and internal walls; the cuticle is thin; stomata are situated above the epidermial cells, the protective hairs and the secretory trichomes are multicellular and one-layered.

The cortex is thin (5-6 layers) (Pl. I: Fig. 10), collemchymatised in subepidermic position, in the ribs.

In comparison with the anterior structure, there is a development of the mechanic tissue (Pl. I: Fig. 10) as well as of the xylemic conductive tissue, from both qualitative and quantitative point of view. The conductive tissues form phloemis-xylemic vascular bundles of medium dimensions, separated by sclerified and lignified multi-layered medullar rays (Pl. I: Fig. 1). The bundles bear cordons of sclerenchymatic fibers at the internal face of the primary xylem and, especially, at the external face of the phloem.

The pith has a big contribution in enlarging the diameter of the stem; it is cellulosed in the internal part and lignified in the external (perimedullar) region (Pl. I: Fig. 7).

Maxiroot 0.4% (V_2). In the following variants we will present only the modifications induced by the variants in work. This time the fascicular type structure has a similar aspect, with the following differences:

- the cortex bears more layers (6-7) (Pl. I: Fig. 2);
- the vascular tissue passes to the annular type, because of the sclerification and lignification of interfascicular medullar rays; the shape of the ring is sinuous, as well as of the cambial tissue which had produced it (Pl. I: Fig. 2);
- the biggest bundles have a thick sheath of sclerenchymatic fibers at their external part, with thicker wall than in V_1 (Pl. I: Fig. 11);
- the pith is thick, parenchymatous, with lignificated perimedullary region (Pl. I: Fig. 6).

Maxiroot 0.6% (V_3). Young stem. The fascicular structure shows the following differences:

V_3 variant indicates the activity of cellular division stimulation, because the diameter of the stem is bigger. The epidermis bears tangentially elongated cells with weaker thickened external and internal walls than in the young stem V_1 (Pl. I: Fig. 5).

The cortex, although well represented (7-8 layers) has a reduced collenchymatic region, visible only in the ribs, where the walls of the cells are weak thickened (Pl. I: Fig. 5). Between the xylem vessels, at their internal pole, numerous cells of cellulosed parenchyma and libriform with thin walls are displayed, while cordons of periphloemic sclerenchyma, although well represented, bear cells with thinner walls than in V_2. The division activity of the cambium is stimulated in this variant (Pl. I: Figs. 5 and 12); at the internal pole, the formation of numerous xylemic rays is in progress. The pith, although better represented than in V_2, is parenchymatous and lignificated only at the external part.

In the mature stem, the cross section has a circular irregular-ribbed profile (Pl. I: Fig. 3), but the ribs are less evident. By the hypodermic cortical layer, phellogen differentiates and produces 2-3 continuous layers of cork which will substitute the epidermis (Pl. I: Fig. 13). The collenchymatic region of the cortex is reduced to 1-3 layers in the ribs and in their lateral parts.

The conductive tissue passes to the annular type due to the sclerification and lignification of the medullar rays, weaker than in the anterior variant (mature stem) (Pl. I: Fig. 12).

Only in the ribs, where the vascular bundles were well developed, there is a thick cordon of mechanic fibers, with moderately thickened walls (Pl. I: Fig. 9).

The pith is thick, parenchymatous, with weak lignified perimedullar region; in the central part, the pith disorganizes itself (Pl. I: Fig. 8).

The blank sample. Although the structure of the species is well known in the specialty literature [METCALFE & CHALK, 1950], for a better understanding of the histological changes, we present only the image with the cross section through the stem, without interpretation, because the structure is similar to that of V_1 from Aurora product. The images are attached to confirm the similarity (Pl. III: Fig. 47-53).

Aurora 0.2% (V_1). The cross section has an elliptic-ribbed profile (Pl. I: Fig. 14).

The epidermis presents cells with external and internal walls thicker than the others (Pl. I: Figs. 14 and 17); the external wall is covered by a thick cellulosed cuticle, which forms a characteristic relief in the ribs. The epidermis shows protective hairs and secretory trichomes, in small number (Pl. I: Fig. 17), while stomata are situated at the same level with the epidermic cells and form a narrow substomatic chamber.

The cortex is thin (6-7 layers) and differentiated into cordons of annular collenchyma, well developed in the ribs, and assimilatory parenchyma in the rest; the most internal layer is a primary endodermis.

The vascular tissues form phloemic-xylemic bundles of collaterally-opened type (Pl. II: Figs. 24 and 29), separated by wide medullar rays, bearing cells with moderately sclerified and lignified walls. The biggest bundles present, at the external part of the phloem, a sheath of sclerenchymatic fibers with weak to moderately-thickened walls and wide lumina. The pith is thick, parenchymatous-cellulosed, formed by very big cells.

The mature stem has a circular-elliptic profile in cross section. The structure is similar to the anterior one, but with a better development of the sustaining tissues (Pl. I: Figs. 21, 25), by getting thickened cellular walls; the effect of these modifications could be seen in the pith which has a wider diameter and it is disorganized. There is an interesting aspect in this variant: in Aurora 0.2% the subepidermic phellogen appears early, especially in the ribs. It forms 1-2 layers of cork at the external part and phellodermis at the internal part.

A primary endodermis is present.

The vascular tissues form big phloemic-xylemic bundles separated by sclerified and lignified multilayered medulary rays (Pl. I: Fig. 14). The bundles have a secondary structure, presenting at the internal face of the primary xylem and especially at the external face of the phloem cordons of sclerenchymatic fibers (Pl. II: Fig. 21). Phloem consists of sieved tubes, guard cells and less parenchyma cells. The primary xylem has perimedullar position, while the secondary xylem is situated near the phloem; the vessels are separated by libriform in the last one (Pl. II: Figs. 28 and 30).

The pith is parenchymatous cellulosed, with tendencies of perimedullary lignifications (Pl. I: Fig. 14; Pl. II: Fig. 20).

Aurora 0.4% (V_2). The cross section of the stem has a circular-ribbed profile, with small ribs (Pl. I: Figs. 15 and 18). V_2 indicates a stimulating activity of the cellular divisions and, as a consequence, an increment in the diameter of the stem (Pl. I: Fig. 15). The cortex consists of 8-10 layers of small cells; it is differentiated into angular collenchyma (Pl. II: Figs. 26 and 27) in the ribs and a parenchymatic region which ends in a primary endodermis (Pl. I: Fig. 18). Although the cellular division is stimulated, the mechanic elements develop normally, being better represented than in V_1.

The vascular tissues form numerous bundles of collaterally-opened type, of bigger dimensions than in the anterior variant (Pl. I: Fig. 18), separated by wide medullar rays formed by cells with moderately sclerified and lignified walls. The xylemic vessels are irregularly disposed, in rows (Pl. II: Fig. 31).

The periphloemic sclerenchyma (Pl. II: Fig. 22) is well developed especially near the biggest vascular bundles (Pl. I: Figs. 15 and 18), while the pith is parenchymatous cellulosed (Pl. I: Fig. 18) with big cells in the central part and smaller near the xylem, where a few thickening tendencies could be observed.

Aurora 0.6% (V_3). The cross section through the stem has circular-ribbed profile (Pl. I: Fig. 16; Pl. II: Fig. 19). The phellogen is differenciated in hypodermic position and generates 2-3 layers of cork at the external part and phelloderm at the internal part (Pl. II: Fig. 32). The cork has various dimensions in the stem circumference.

The cortex consists of 4-5 layers of cells with cellulosed walls (Pl. II: Fig. 19).

The conductive tissues form 2 concentric rings (Pl. II: Fig. 19), with quite similar width, one of them is the phloem, situated at the external part and the other is the xylem, at the internal part, bearing big quantities of libriform. Only near the biggest vascular bundles, thick cordons of sclerenchymatic fibers are present, with big lumina and thick walls (Pl. II: Fig. 23).

The pith is thick, parenchymatous, meatous type, bearing big cells.

Dacmarinur 0.2% (V_1). The cross section through the stem has a circular elliptic-ribbed shape, with 4 prominent ribs (Pl. II: Fig. 33).

The epidermis consists of cells with thickened external and internal walls (Pl. III: Fig. 42), the external one being covered by thin cuticle. The cortex is thin and consists of 4-5 layers of cells (Pl. II: Fig. 33); collenchymatous in the ribs and parenchymatous-assimilatory in the rest; the collenchyma of the ribs is quite weak represented (Pl. III: Fig. 42). The endodermis is present (Pl. III: Fig. 39).

The conductive tissues form numerous vascular bundles of collaterally-opened type, the biggest bundles alternate with the smallest ones (Pl. II: Fig. 33; Pl. III: 43), the first mentioned have a cordon of mechanic fibers with thin walls (Pl. III: Fig. 39), with the process of lignification in progress and wide lumina. The vascular bundles are separated by wide parenchymatic medullar rays. The phloem consists of sieved tubes, guard cells and a few parenchymatic cells, while the xylem consists of vessels disorderly disposed, separated by cellulosed parenchyma; only near the phloem, between the xylem vessels, a few libriform elements could be observed, with thin walls (Pl. III: Fig. 43). The pith is thick and cellulosed.

Dacmarinur 0.4% (V_2). The cross section through the stem has a circular-ribbed shape (Pl. II: Fig. 34). The phellogen is differentiated in hypodermic position and forms 1-2 layers of cork at the external part and phelloderm at the internal one (Pl. III: Fig. 46).

The cortex consists of 7-9 layers of cells (Pl. II: Fig. 36).

The conductive tissues (Pl. II: Figs. 34 and 36) form big vascular bundles, separated by multi-layered sclerified and lignified medullar rays (Pl. II: Fig. 36; Pl. III: 44). The bundles present secondary structure, bearing at both the internal face of the primary xylem and, especially, at the external face of the phloem seaths of sclerenchymatic fibers with thick walls and narrow lumina. The phloem consists of sieved tubes, guard cells and a few phloemic parenchyma, while the xylem consists of vessels disorderly disposed in the fundamental libriform and lignified xylemic parenchyma (Pl. III: Fig. 40).

The pith is thick; the cells from the center are in disorganizing process, ending in aeriferous cavity; the cells near the xylem (perimedullar region) lignify their walls (Pl. III: Fig. 38).

Dacmarinur 0.6% (V_3). The cross section through the stem has a circular-ribbed shape, bearing attenuated ribs (Pl. II: Fig. 35).

The phellogen is differentiated in subepidermic position and forms 1-2 layers of cork at the external part and phelloderm at the internal one (Pl. III: Figs. 37 and 45).

The cortex has smaller dimensions in comparison with the anterior variant (5-6 layers of cells); it ends in a primary endodermis (Pl. III: Figs. 37 and 45).

The conductive tissue is somehow developed at a level between V_1 and V_2 (Pl. II: Fig. 35). The bundles are separated by wide multi-layered, sclerified and lignified medullar rays (Pl. III: Fig. 37). The components of the xylem and phloem are similar as in the anterior variants, with the difference that in V_3 the cordons of periphloemic sclerenchyma are less numerous, only in the ribs; they have moderately thickened walls and wide lumina (Pl. III: Fig. 41).

The same tendencies of disorganization appear in the central part of the pith, as in the anterior variant (V_2).

PLATE I

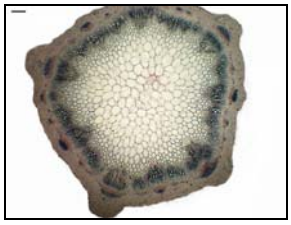

Fig. 1

Fig. 2

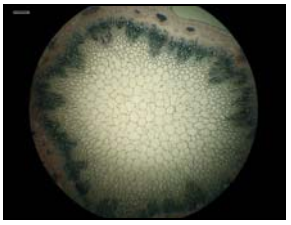

Fig. 3

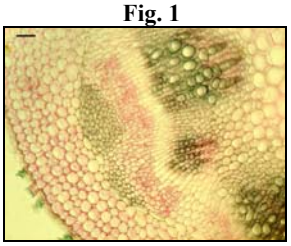

Fig. 4

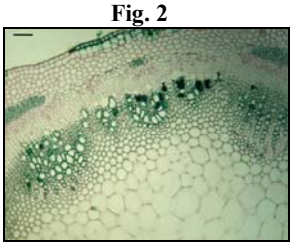

Fig. 5

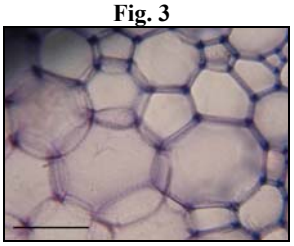

Fig. 6

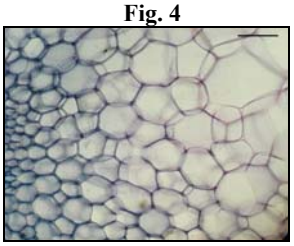

Fig. 7

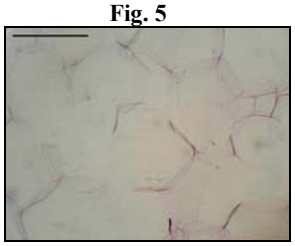

Fig. 8

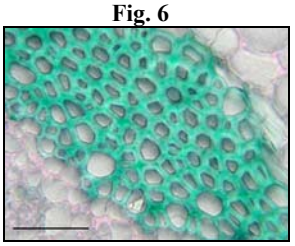

Fig. 9

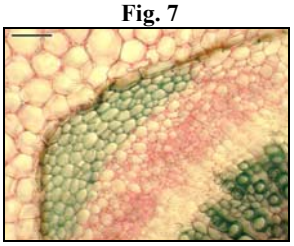

Fig. 10

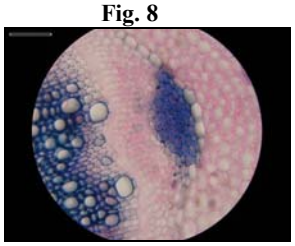

Fig. 11

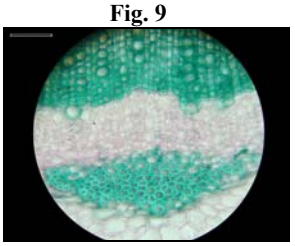

Fig. 12

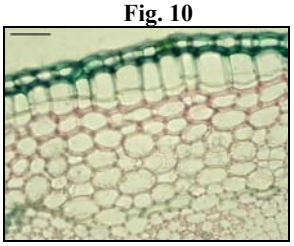

Fig. 13

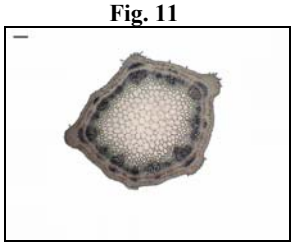

Fig. 14

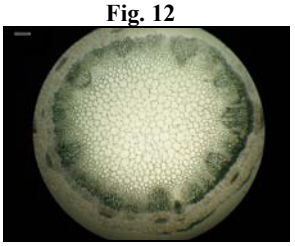

Fig. 15

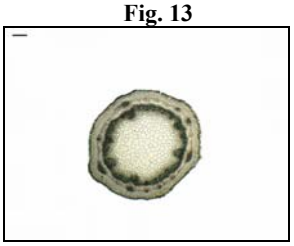

Fig. 16

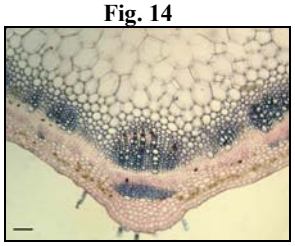

Fig. 17

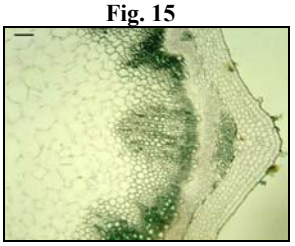

Fig. 18

PLATE II

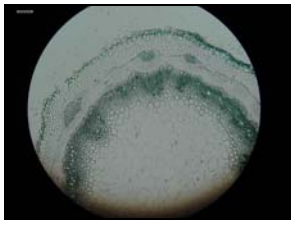

Fig. 19

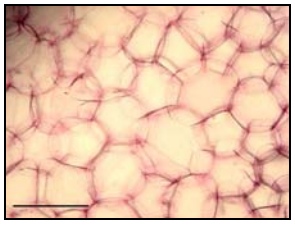

Fig. 20

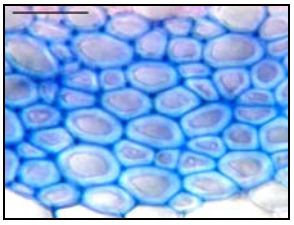

Fig. 21

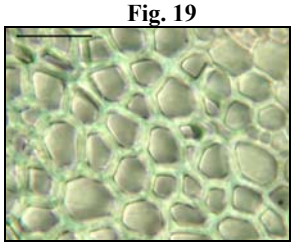

Fig. 22

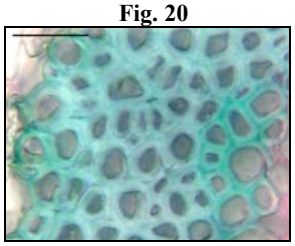

Fig. 23

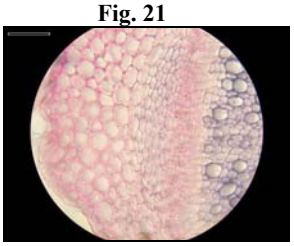

Fig. 24

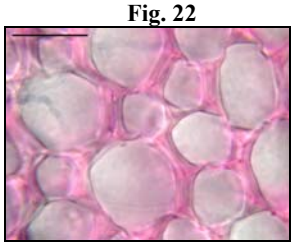

Fig. 25

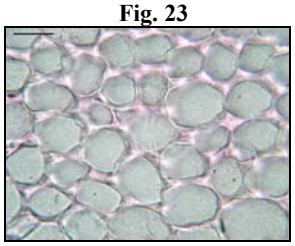

Fig. 26

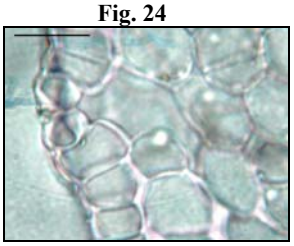

Fig. 27

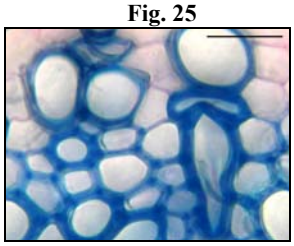

Fig. 28

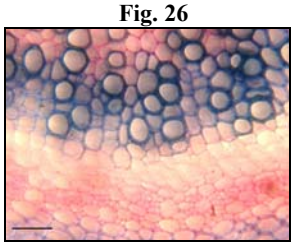

Fig. 29

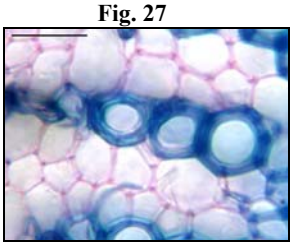

Fig. 30

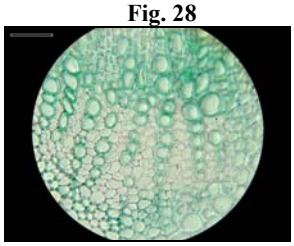

Fig. 31

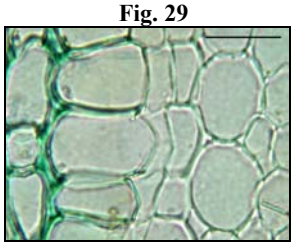

Fig. 32

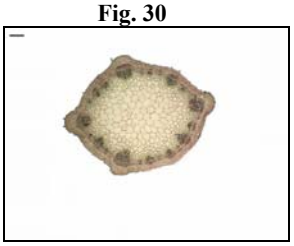

Fig. 33

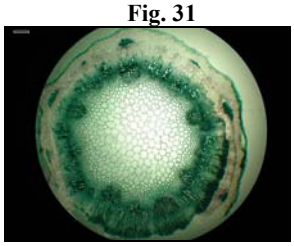

Fig. 34

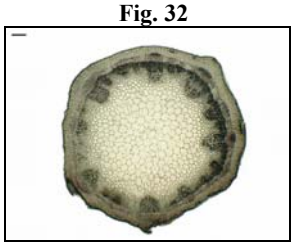

Fig. 35

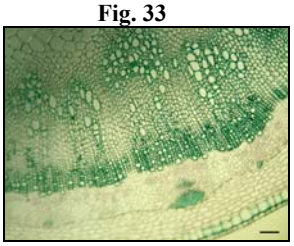

Fig. 36

PLATE III

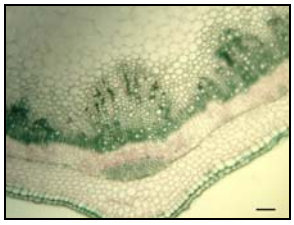

Fig. 37

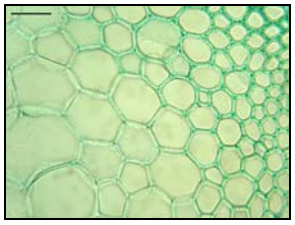

Fig. 38

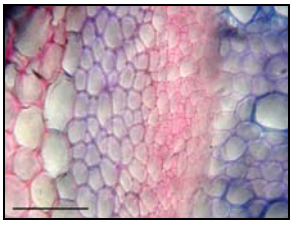

Fig. 39

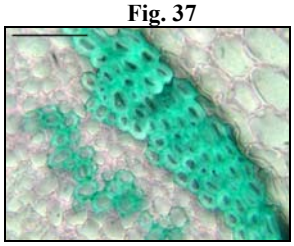

Fig. 40

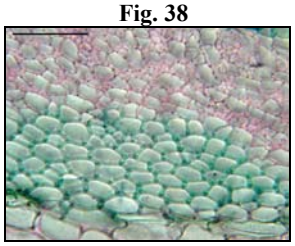

Fig. 41

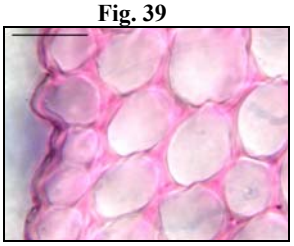

Fig. 42

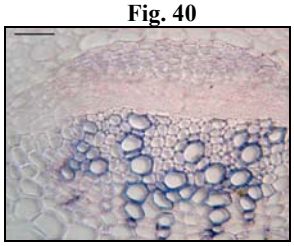

Fig. 43

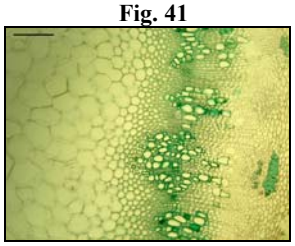

Fig. 44

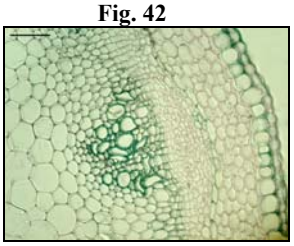

Fig. 45

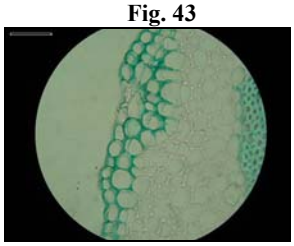

Fig. 46

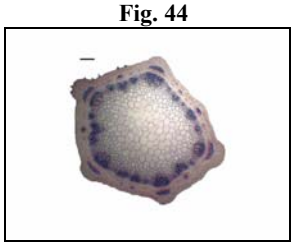

Fig. 47

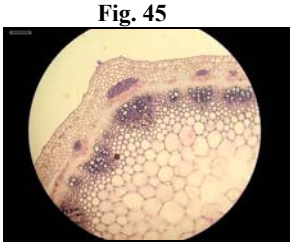

Fig. 48

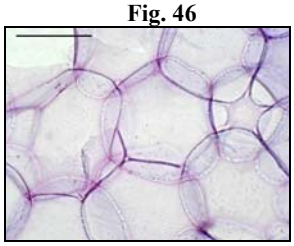

Fig. 49

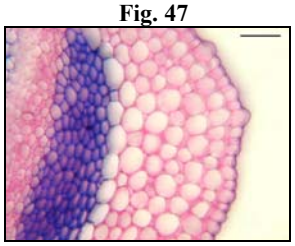

Fig. 50

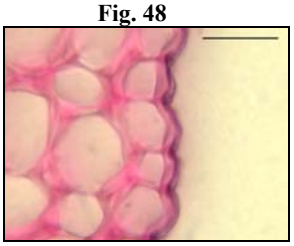

Fig. 51

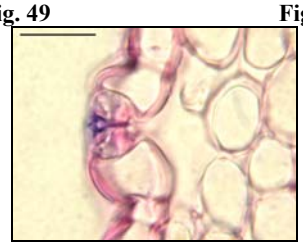

Fig. 52

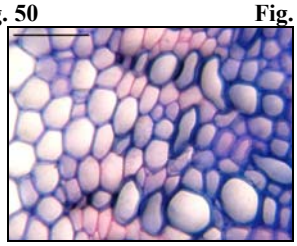

Fig. 53

Conclusions

In the plants treated with Maxiroot 0.2% there is a weak increment of the stem diameter, while the sclerification and lignification is advanced in comparison with the blank sample. In the plants treated with Maxiroot 0.4%, a strong development of the conductive tissue, from both qualitative and quantitative point of view, is displayed; the periphloemic sclerenchyma is more developed in comparison with the above variant. In V_3, although the diameter of the stem is the biggest, the collenchymatisation, sclerification and lignification of the tissues are weaker than in V_2. The comparative analysis of the young and mature stems in V_3 indicates an intensification of the cellular divisions which guides to an increment of the diameter. Due to some disruptions observed in the tissues, especially in the pith, we do not recommend the usage of V_3.

In the plants treated with Aurora, V_2 is superior to the others variants, V_1 and V_3. At 0.2%, although the structure is similar to that of the blank sample (the stem has a narrow diameter), the mechanic elements are well developed. Aurora can be successfully applied in all variants, although we recommend the variant 0.4%.

In the plants treated with Dacmarinur, in 0.4%, the diameter of the stem is big; this is a positive reaction because the tissues are well developed, while the cellular walls are thickened; the effect of the concentration 0.6% is also positive.

For all products, the modifications appeared in V_2 were insignificant in comparison with the blank sample, but also in a comparison between various products; in the other variants an activation of the cellular divisions is initiated. In Dacmarinur Max. N there is a strong stimulation in comparison with the other products.

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Explanation of plates

PLATE I. Details of the structure of the stems belonging to the plants treated with Maxiroot, in various concentrations (Figs. 1-13). Details of the structure of the stems belonging to the plants treated with Aurora, in various concentrations (Figs. 14-18):
Fig. 1. Maxiroot V_1
Fig. 2. Maxiroot V_2
Fig. 3. Maxiroot V_3
Fig. 4. Maxiroot V_1
Fig. 5. Maxiroot V_3
Fig. 6. Maxiroot V_2
Fig. 7. Maxiroot V_1
Fig. 8. Maxiroot V_3
Fig. 9. Maxiroot V_3
Fig. 10. Maxiroot V_1
Fig. 11. Maxiroot V_2
Fig. 12. Maxiroot V_3
Fig. 13. Maxiroot V_3
Fig. 14. Aurora V_1
Fig. 15. Aurora V_2
Fig. 16. Aurora V_3
Fig. 17. Aurora V_1
Fig. 18. Aurora V_2

PLATE II. Details of the structure of the stems belonging to the plants treated with Aurora, in various concentrations (Figs. 19-32). Details of the structure of the stems belonging to the plants treated with Dacmarinur, in various concentrations (Figs. 33-36):
Fig. 19. Aurora V_3
Fig. 20. Aurora V_1
Fig. 21. Aurora V_1
Fig. 22. Aurora V_2
Fig. 23. Aurora V_3
Fig. 24. Aurora V_1
Fig. 25. Aurora V_1
Fig. 26. Aurora V_2
Fig. 27. Aurora V_2
Fig. 28. Aurora V_1
Fig. 29. Aurora V_1
Fig. 30. Aurora V_1
Fig. 31. Aurora V_2
Fig. 32. Aurora V_3
Fig. 33. Dacmarinur V_1
Fig. 34. Dacmarinur V_2
Fig. 35. Dacmarinur V_3
Fig. 36. Dacmarinur V_2

PLATE III. Details of the structure of the stems belonging to the plants treated with Dacmarinur, in various concentrations (Figs. 37-46). Details of the structure of the stems belonging to the blank samples (Figs. 47-53):
Fig. 37. Dacmarinur V_3
Fig. 38. Dacmarinur V_2
Fig. 39. Dacmarinur V_1
Fig. 40. Dacmarinur V_2
Fig. 41. Dacmarinur V_3
Fig. 42. Dacmarinur V_1
Fig. 43. Dacmarinur V_1
Fig. 44. Dacmarinur V_2
Fig. 45. Dacmarinur V_3
Fig. 46. Dacmarinur V_2
Fig. 47. Blank sample
Fig. 48. Blank sample
Fig. 49. Blank sample
Fig. 50. Blank sample
Fig. 51. Blank sample
Fig. 52. Blank sample
Fig. 53. Blank sample