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The effects of some cinnamic acid derivatives on the architecture of *Phaseolus vulgaris* roots

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Abstract

The influence of ten cinnamic acid derivatives (cinnamic acid, 4-methoxi-cinnamic acid, 4-(N,N-dimethylamino)-cinnamic acid, ferulic acid, 3,4-dimethoxi-cinnamic acid, p-coumaric acid, 4-methyl-cinnamic acid, 4-chloro-cinnamic acid, 3-bromo-cinnamic acid, caffeic acid) on the development and histo-anatomy of Phaseolus vulgaris root was investigated. The plants were grown on an artificial substrate impregnated with 114.6 µg/cm^2 active compound. The transverse sections through the roots of the treated plants were compared to those obtained from the control, revealing the fact that most of the tested chemicals inhibited the formation of root hairs and induced changes in the structure of the vascular system. The phloem elements were the most affected. The most interesting alterations were recorded for 4-methyl-cinnamic acid, the chemical treatment causing the appearance of atypical vessels in the area of transition from root to hypocotyl. The results confirmed the phytotoxic potential of the investigated chemicals, stressing the importance of further toxicological evaluation for these compounds and their analogues, if they are to be used for their antimicrobial and antioxidant properties, but also making them good candidates for the development of new herbicides.

Keywords: histomorphological evaluation, phytobiological tests, root hairs, vascular tissues.

Introduction

Using plants as test organisms for toxicity evaluation has proved to be very useful both in the field of fundamental research for developing new drugs, as well as in environmental monitoring. Plant experiments present the advantages of being easy to handle, low cost and of having good correlation with other tests. Therefore, these tests can represent valuable screening tools; positive results in phytobiological tests should be regarded as an indication that the investigated substances may be a biological hazard in other superior organisms as well [1].

One of the plant species recommended by the Food and Drugs Administration (FDA) for phytotoxicity tests is *Phaseolus vulgaris*, many studies using different species within the *Phaseolus* genus [2-9].

In our previous research we used *Phaseolus* test to assess the inhibitory effect of some cinnamic acid derivatives, which exhibited antimicrobial properties, on the growth of bean plants and on the biosynthesis of polyphenols, as markers of the plants' metabolism. The most affected by the chemical treatments was the growth of the roots, in comparison with the seedlings'. Three active concentrations of substances were used (28.6 µg/cm^2, 57.3 µg/cm^2 and 114.6 µg/cm^2), the most extensive alterations appearing at the highest tested concentration [10], this being the reason why we chose for histomorphological evaluation the roots of the plants developed at 114.6 µg/cm^2.

Materials and methods

Phaseolus vulgaris seeds obtained in 2011 from an experimental plot located at Ezăreni (the research farm within the University of Agricultural Sciences and Veterinary Medicine Iasi) were grown on an artificial substrate (WHATMAN® no.1 filter paper) impregnated with different dilutions of the tested compounds and the development of the resulting seedlings was observed in comparison to a control group, represented by plants resulted from seeds displayed on a simple filter paper. Ten cinnamic acid derivatives were used in the study: cinnamic acid, 4-methoxi-cinnamic acid, 4-(N,N-dimethylamino)-cinnamic acid, ferulic acid, 3,4-dimethoxi-cinnamic acid, p-coumaric acid, 4-methyl-cinnamic acid, 4-chloro-cinnamic acid, 3-bromo-cinnamic acid, caffeic acid. The substances, with the exception of caffeic acid (purchased from FLUKA), were synthesized and their structures were confirmed by spectral data and quantitative elemental analysis [11]. Three active concentrations were tested for each substance and the concentration of active compound was expressed in $\mu g/cm^2$: 28.6 $\mu g/cm^2$; 57.3 $\mu g/cm^2$; 114.6 $\mu g/cm^2$ [10].

The histoanatomical changes induced in the meristematic tissue of bean roots under the influence of the synthesized compounds were observed only for the plants exposed to the highest tested concentration (114.6 $\mu g/cm^2$). The plant material was first fixed and preserved in ethylic alcohol 70%. The sections were cut manually, using the microtome. The histological sections were collected on a glass watch with distilled water. The sections were coloured with iodine green (1 minute), washed in 90% ethylic alcohol and distilled water then coloured with ruthenium red (1 minute) and again washed in distilled water. The lignified or suberized cell walls appeared colored in green, in contrast with the cellulosic ones that were red [12].

In order to obtain permanent slides, the histological sections were mounted in glycerol-gelatine drops; then they were analyzed with an Optika light microscope. The light micrographs were performed using a Canon A540 camera [13]. Transverse sections through *Phaseolus vulgaris* plants were performed at two levels: the apical part of the root and the transition area from root to hypocotyl.

Results and Discussions

For the **control plants**, the root presented the well-known anatomic regions. Three main zones were identified in the transverse sections at the apical part of the root: the rhizodermis, cortex and the stele. The rhizodermal cells were regular in shape or slightly tangentially elongated, their size varying. The rhizodermis presented numerous root hairs, with thin, cellulosic walls.

The cortex region was well developed, very thick, divided in three subregions (exodermis, cortical parenchyma and endodermis). The exodermis contained 1-2 layers of cells, much larger than the rhizodermal cells and with thicker, suberized walls. The cortical parenchyma had 12-14 layers of round cells, separated by intercellular spaces, rich in starch, their size decreasing towards the stele. Considering the fact that the root was in the primary state of growth, the endodermis had isodiametric cells, with walls having Casparian strips.

These findings are in accordance with the general knowledge about the architecture of root systems. In young roots, the epidermis is a specialized absorbing tissue containing root hairs, which are themselves specialized projections from modified epidermal cells and the

cortex usually occupies the largest volume of most roots and consists mainly of highly vacuolated parenchyma cells with intercellular spaces between [14].

The central stellar region was relatively thick, with a tetrarch structure [15, 16], presenting 4 poles of xylem, alternating with 4 poles of phloem, separated by thin medullary rays, attached to the pericycle. A relative thin layer of phloem fibers, polygonal-shaped, with small diameters and moderately thickened, lignified walls was observed at the periphery of each phloem pole.

At this stage of development, some large secondary xylem vessels had already formed close to the phloem bundles. The walls of these vessels were still thin, but lignified. The phloem bundles were formed of sieve tubes and companion cells, presenting also a thin layer of phloem fibers. The xylem bundles were approximately trigonal-shaped in transverse section, presenting protoxylem and metaxylem vessels, with thickened and intensely lignified walls.

The pith was well represented at this stage of development (primary structure, the initiation of secondary structure). The medullary cells were larger than the cells in the cortex (Fig 1).

In the transverse section of the area of transition from root to hypocotyl three main regions were also identified: the epidermis, cortex and the stele.

The epidermis was partially exfoliated and some layers of suber had formed in those affected areas. Sparse tricellular hairs were observed between the regular epidermal cells.

The cortex at this level was relatively thick, the last inner layer being represented by the endodermis, presenting cells with Casparian strips.

The central cylinder was also thick, containing discontinuous rings of phloem elements (sieve tubes and companion cells) and of cambium (that had already produced some secondary xylem and phloem elements) and also an internal ring of xylem, formed of protoxylem and metaxylem vessels and of parenchymatous cells with lignified walls. The pith was prominent in the hypocotyl (Fig 2). A similar collateral arrangement of vascular tissues in the upper part of the hypocotyl, with vascular bundles arranged in a ring, was also reported for lignosus bean (*Dipogon lignosus*) [17].

The formation of first-order lateral roots was also observed at this level, with phloem and xylem elements generated by the activity of the pericycle.

The substances that were analyzed in order to assess their phytotoxic potential had modified the histo-anatomy of the roots in the treated plants in comparison with the control.

Cinnamic acid (1)

The main differences that appeared in the transverse section through the root consisted in histomorphological changes of the vascular bundles. The four bundles of primary xylem were not present anymore, the secondary xylem vessels had a large diameter and the tetrarch structure was evident only because of the four bundles of phloem, connected between them with a cord of fibers. Relatively thick primary phloem fibers were present at this level. The development of root hairs on the rhizodermis was not affected by the treatment with cinnamic acid (Fig 1).

The transverse sections through the area of transition from root to hypocotyl revealed two concentric circles of conducting tissues (mainly secondary xylem and phloem); the xylem ring was thicker and it surrounded the pith area. First-order lateral roots had already formed and even second-order ones started to appear (Fig 2).

4-Methoxi-cinnamic acid (2)

4-Methoxi-cinnamic acid inhibited the formation of root hairs on the surface of the rhizodermis, the exodermis being completely sclerified. The conducting tissues were arranged

in two concentric circles, the xylem being totally lignified, with a few secondary xylem vessels. The tested compound also inhibited the development of primary phloem fibers cords; only a few elements with lignified walls were observed near the pericycle (Fig 1, 2).

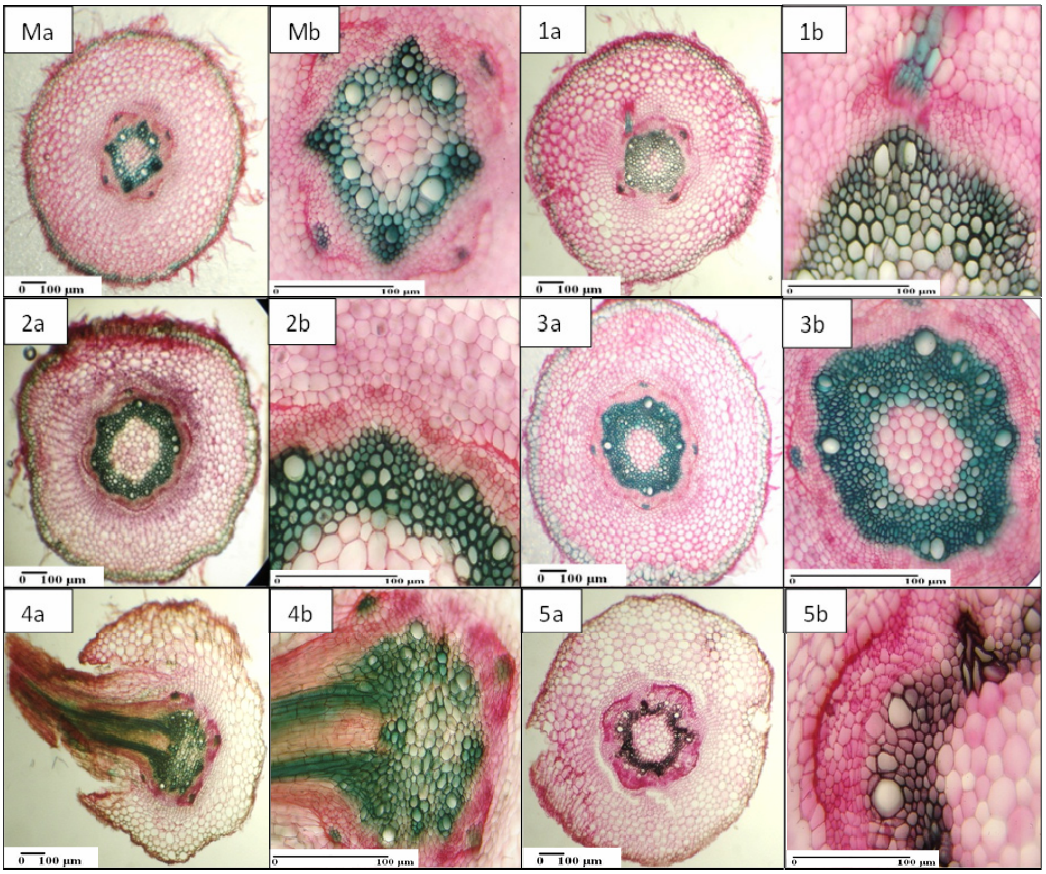

Fig. 1. Transerse sections through the main root of *Phaseolus vulgaris* control and treated plants – whole sections (a) and magnified portions (b)
M: control; **1**: cinnamic acid; **2**: 4-methoxi-cinnamic acid; **3**: N,N-dimethylamino-cinnamic acid; **4**: ferulic acid; **5**: 3,4-dimethoxi-cinnamic acid.

N,N-dimethylamino-cinnamic acid (3)

N,N-dimethylamino-cinnamic acid caused several changes in the root structure of the treated plants. The root hairs' density was considerably lower, the exodermis being sclerified. The xylem ring was thick, some vessels having a large diameter. In contrast, the phloem fibers cords were very thin (Fig 1).

The section of the area of transition from root to hypocotyl appeared to be asymmetrical due to the formation of lateral roots. The appearance of these roots also modified the aspect of the xylem ring. No root hairs were observed on the surface of the rhizodermis and even some scattered primary suber elements were present (Fig 2).

Ferulic acid (4)

The general aspect of the tranverse section of the apical part of the root in plants developed in the presence of ferulic acid was modified due to the penetration of very thick

first-order lateral roots. The beginning of the transition from primary to secondary structure was evident at this level. Several large diameter xylem cells and the initiation of the pith's lignification process were observed. Phloem fibers cords were present at the periphery of each primary phloem bundle (Fig 1).

The contour of the transverse section through the area of transition from root to hypocotyl was influenced by the formation of four first-order lateral roots. The structure of the conducting tissues in the stele combined both primary and secondary xylem and phloem elements, the xylem ring being much thicker. The treatment with ferulic acid inhibited the development of primary phloem fibers at this level (Fig 2).

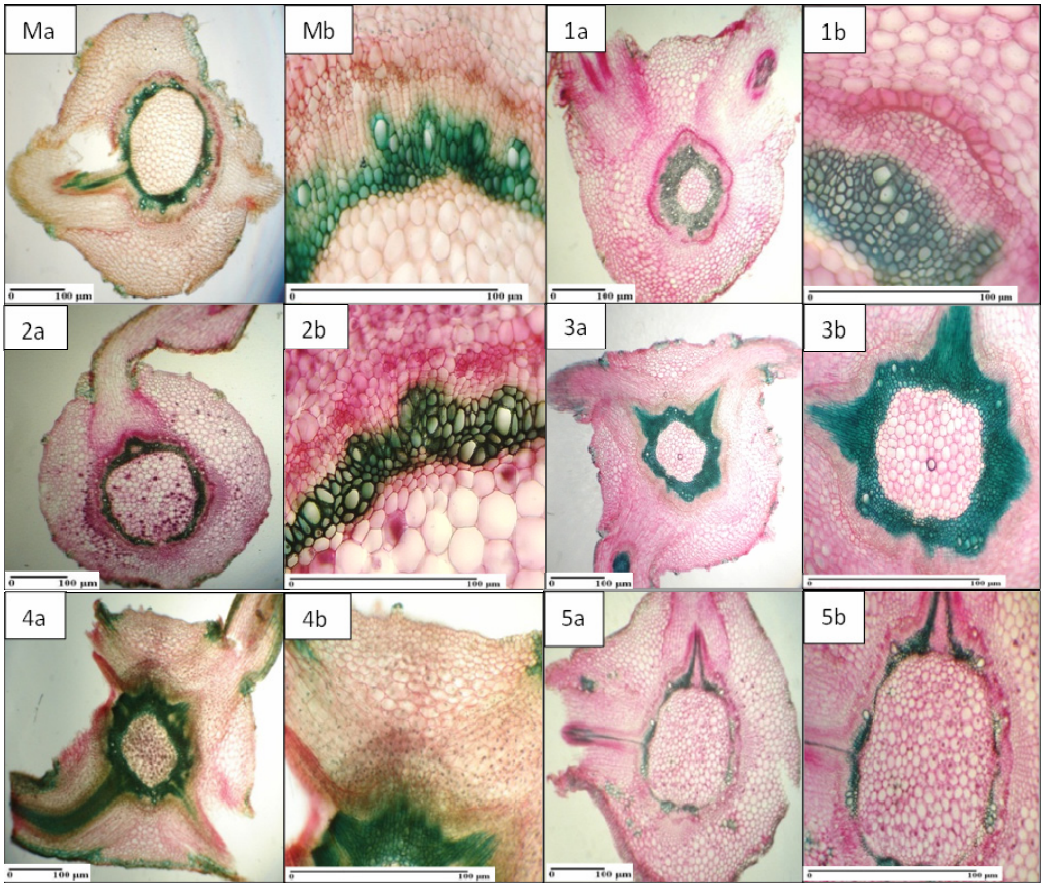

Fig. 2. Transerse sections through the area of transition from root to hypocotyl of *Phaseolus vulgaris* control and treated plants – whole sections (a) and magnified portions (b)
M: control; **1**: cinnamic acid; **2**: 4-methoxi-cinnamic acid; **3**: N,N-dimethylamino-cinnamic acid; **4**: ferulic acid; **5**: 3,4-dimethoxi-cinnamic acid.

3,4-Dimethoxi-cinnamic acid (5)

3,4-Dimethoxi-cinnamic acid induced changes in the histomorphology of the roots. Similar to other cinnamic acid derivatives, the root hairs and the vascular tissues were the affected ones. Only some remainings of root hairs were observed on the rhizodermis at the apical part of the root and the phloem fibers cords were missing at this level (Fig 1).

The structure of the area of transition from root to hypocotyl was characterized by the formation of thick lateral roots and by the absence of absorbent root hairs. The vascular tissues were not very well represented at this level. The xylem vessels appeared grouped in insular formations, rather than forming the xylem ring like in the control plants. The tested substance completely inhibited the development of the phloem fibers cords (Fig 2).

p-Coumaric acid (6)

This cinnamic acid derivative did not induce important modifications in the root structure of *Phaseolus* plants. The formation of root hairs and vascular bundles was not affected. The xylem vessels were displayed in a rhomboid form in the transverse section through the apical part of the root and the primary phloem fibers cords were also visible at this level (Fig 3, 4).

4-Methyl-cinnamic acid (7)

The treatment with 4-methyl-cinnamic acid did not inhibit the formation of root hairs, these elements being frequent in the transverse section of the apical part of the root. The central cylinder was relatively thick at this level, presenting phloem fibers cords and very large xylem secondary vessels (Fig 3).

Major differences in comparison with the control appeared in the transverse section through the area of transition from root to hypocotyl. The topography of the conducting vascular tissues was altered. Due to the transition from primary to secondary structure, the primary xylem and phloem bundles were absent, but also the xylem ring found in all the other investigated cases could not be identified anymore. Instead, cords or isles of lignified xylem and parenchymal elements appeared in the parenchymal mass. The secondary xylem ring was discontinous and it incorporated isles of cellulosic parenchyma. In addition, treatment with 4-methyl-cinnamic acid initiated a cell division process localized mainly in the xylem, but also affecting the phloem. The resulting meristematic tissue was not organized in a circular structure (to form a ring), but instead it formed isles in various locations, which produced either both types of conductiving elements (xylem and phloem) or only xylem, the resulting vessels being atypical (Fig 4).

4-Chloro-cinnamic acid (8)

Several histoanatomical changes were induced by 4-chloro-cinnamic acid in the roots of *Phaseolus vulgaris* plants. In the transverse section of the apical part of the root, the pith area appeared diminished in comparition with the control. Mainly, primary xylem vessels were observed, the bundles being separated by large medullary rays. The phloem fibers cords found in the control at the periphery of the primary phloem bundles were absent in plants developed in the presence of 4-chloro-cinnamic acid (Fig 3).

The structure of the area of transition from root to hypocotyl appeared asymmetric in the transverse section, the absorbent hairs were absent and the cortex was thick and about to be penetrated by lateral roots. The thickness of the cambial and of the xylem ring varied in the stele. The pith was also thick at this level (Fig 4).

3-Bromo-cinnamic acid (9)

At the apical part of the root, in transverse section, a high number of root hairs were observed. The formation of root nodules was initiated at this level by the penetration of rhizobia into the cortex. The central cylinder appeared square-shaped, with four phloem bundles in each corner, presenting thick cords of primary sclerenchyma fibers at the periphery. The xylem bundles had trigonal shapes; some secondary xylem vessels that would

later replace the medulla were also present. Very few medullary parenchymal cells, with lignified walls, still persisted at this level (Fig 3).

The developement of root hairs on the rhizodermis in the area of transition from root to hypocotyl was inhibited by the treatment with 3-bromo-cinnamic acid. Two concentric rings of vascular tissues, xylem and phloem, both primary and secondary, were visible in the central cylinder. The first order lateral roots reached the surface of the investigated organ, presenting a thick and completely lignified xylem cord (Fig 4).

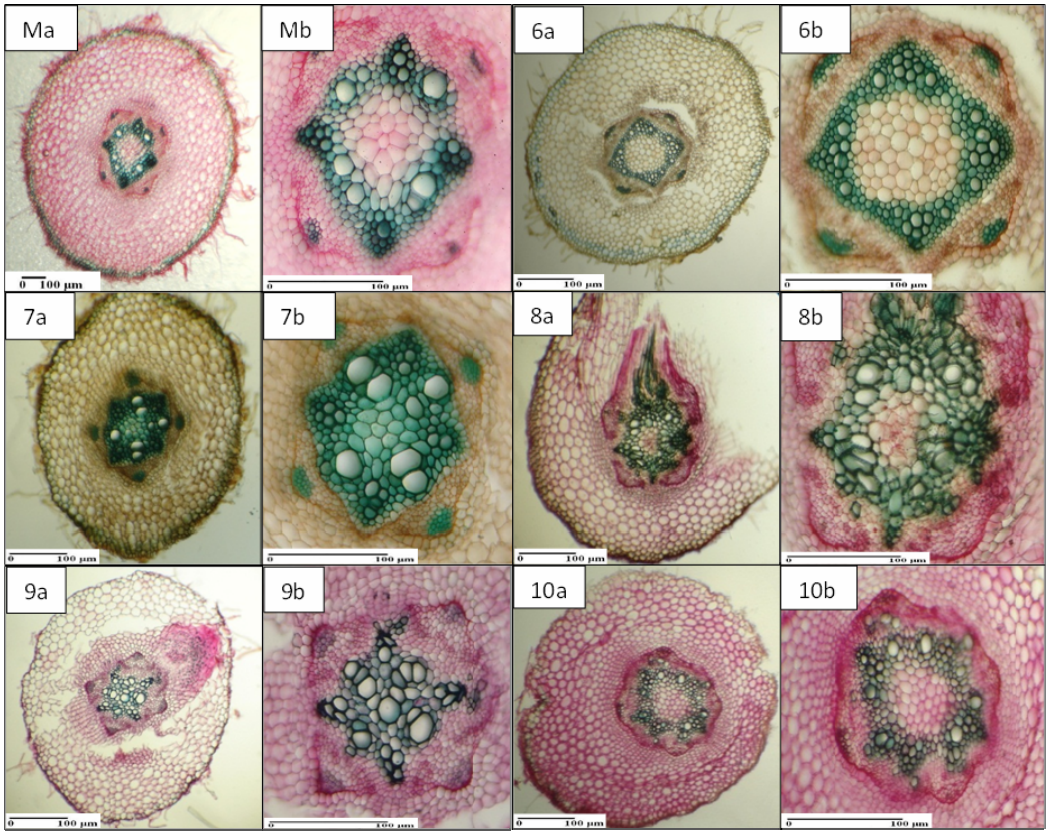

Fig. 3. Transerse sections through the main root of *Phaseolus vulgaris* control and treated plants – whole sections (a) and magnified portions (b)
M: control; **6**: p-coumaric acid; **7**: 4-methyl-cinnamic acid; **8**: 4-chloro-cinnamic acid; **9**: 3-bromo-cinnamic acid; **10**: caffeic acid.

Caffeic acid (10)

The structure of the apical part of the root in plants treated with caffeic acid was, in general, similar to that of the control, yet the formation of root hairs was affected, the density of root hairs being lower than for chemically untreated plants. The xylem ring contained very few secondary xylem vessels and the phloem fibers cords appeared very thin (Fig 3).

The structure of the area of transition from root to hypocotyl was characterized by the presence of very thick lateral roots and of a discontinuos ring of xylem elements, absent in some areas, but better represented in the region next to lateral roots' formation place. No primary phloem fibers were identified at this level. The pith was very thick (Fig 4).

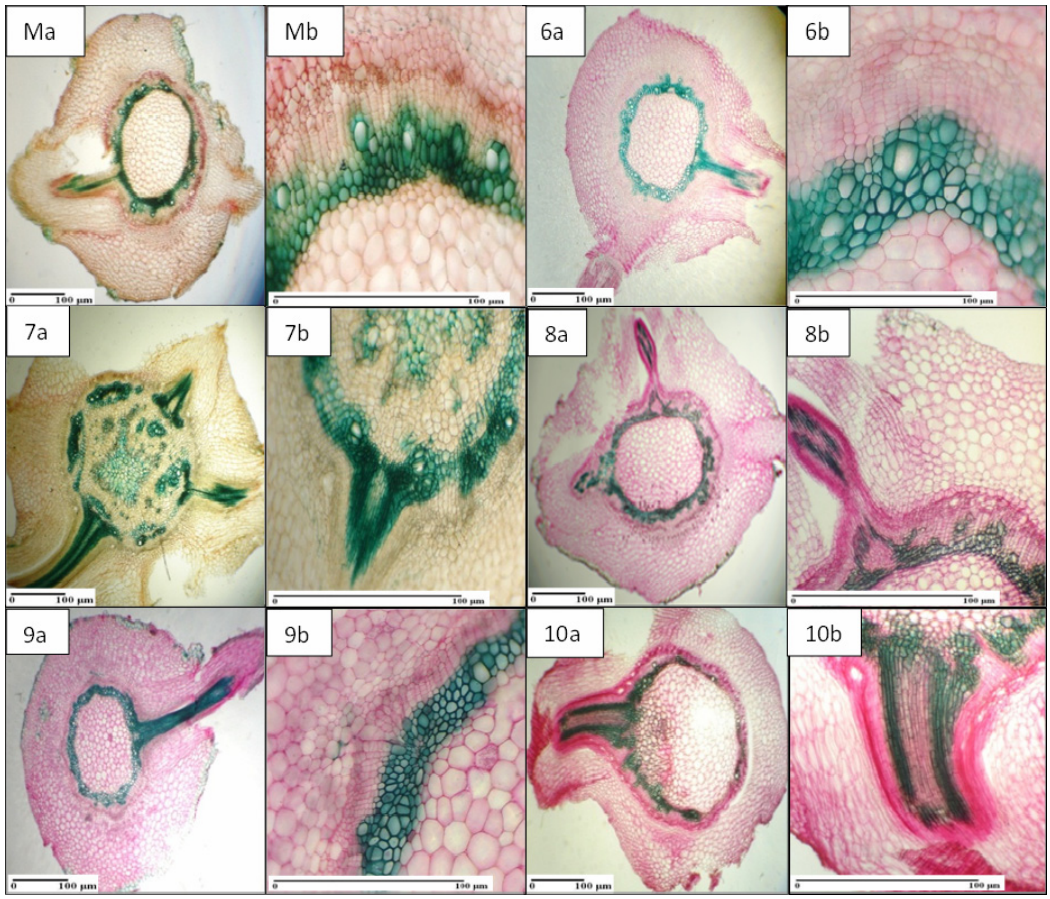

Fig. 4 .Transerse sections through the area of transition from root to hypocotyl of *Phaseolus vulgaris* control and treated plants – whole sections (a) and magnified portions (b)
M: control; **6**: p-coumaric acid; **7**: 4-methyl-cinnamic acid; **8**: 4-chloro-cinnamic acid; **9**: 3-bromo-cinnamic acid; **10**: caffeic acid.

The toxic effects of cinnamic acid derivatives appeared usually in the form of inhibition of root hairs and changes induced in the structure of vascular bundles. The most affected were the phloem elements. Similar results were also obtained by Azmat *et al* [2] when they examined the toxic effects of Pb on *Phaseolus mungo* and by Singh *et al* [6], the second team of researchers investigating the arsenic-induced root growth inhibition in *Phaseolus aureus*.

Root hairs have two main functions: the absorption of water and mineral ions from soil and adhesive properties between root and the surroundings [2]; their formation is a complex process regulated by many genes and is also responsive to a variety of environmental stimuli [14, 18]. One of the plant hormones that influences the development of root hairs is auxin [18]. Auxin is a potent hormonal effector of root hair development. Some studies have revealed that aberrations in auxin availability or signaling can cause defects in root hair growth and morphology [19].

Trans cinnamic acid is a potent auxin-inhibitor [20], therefore this mechanism of action could explain the interference of the tested compounds with the formation and development of root hairs.

If the inhibitory effect of the investigated chemicals on root hairs can be associated with the anti-auxin potential of the compounds, the same reasoning could not be used to explain the changes induced in the structure of vascular system, because the most affected were the phloem vessels. The vascular tissues are induced and controlled by auxin, but in tissue cultures low auxin concentrations induced sieve elements, but not tracheary elements, whereas high auxin concentrations resulted in the differentiation of both phloem and xylem [21].

All in all, root hairs and vascular tissues are responsible for the absorption and conduction of water and nutrients. Due to the inhibitory effect of the compounds on the development of root hairs and phloem tissues, the conduction of water, minerals and nutrients was affected, which ultimately led to growth reduction (of both roots and seedlings) and changes in morphology of plants, as we showed in our previous work. For six of the investigated substances (3,4-dimethoxi-cinnamic acid, 4-methoxi-cinnamic acid, 4-chloro-cinnamic acid, 3-bromo-cinnamic acid, 4-(N,N-dimethylamino)-cinnamic acid and 4-methyl-cinnamic acid) we found percentages of inhibition for root growth higher than 70% at a concentration of 114.6 $\mu g/cm^3$ [10]. These percentages were in accordance with the extent of the histomorphological changes found in the roots of the treated plants.

These results are also in agreement with the data previously published. Caffeic, p-coumaric, ferulic and cinnamic acid are known potent root growth inhibitors [22, 23]; furthermore, caffeic acid affects early growth and morphogenetic response of hypocotyl cuttings of mung bean, interfering also with the rooting potential [4].

Conclusions

In conclusion, the phytotoxicity of the compounds also manifested on the roots' architecture of *Phaseolus vulgaris* plants. The most interesting modifications were recorded for 4-methyl-cinnamic acid, the chemical treatment causing the appearance of atypical vessels in the area of transition from root to hypocotyl. These supplementary data strengthen the idea that the toxicity of the tested substances on living organisms must not be ignored, if the substances or their analogues are considered to be used in therapy for their antimicrobial and antioxidant properties. Furthermore, the phytotoxic potential of these chemicals makes them good candidates for the development of new herbicides.

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