RESEARCHES REGARDING MICROFLORA ON SOME PERENNIAL ORNAMENTAL PLANTS

CERCETĂRI PRIVIND MICROFLORA CARE SE DEZVOLTĂ PE UNELE PLANTE ORNAMENTALE PERENE

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Abstract. *Researches were carried out on two species of perennial ornamental plants, Thuja orientalis L. (commonly known as tree of life or thuja) and Buxus sempervirens L. (common names include box or boxwood) cultivated in the greenhouse of the Didactic Station Iasi. Anatomical and physiological characters of the root and leaf surface and their physic -chemical environments substantially influence the density and diversity of phyllosphere- and rhizosphere-inhabiting microorganisms, which may include natural antagonists of important pathogens. The objective of this investigation was to quantify the phyllosphere (i.e. leaf surface) and rhizosphere (i.e. root surface) microbial population from two species of perennial ornamental plants and to identify the genus of fungi for a better differentiation between species and for the same species between the rhizosphere and phyllosphere.*

Rezumat. *Cercetările au fost efectuate pe plante ornamentale perene de tuia (Thuja orientalis L.) şi buxus (Buxus sempervirens L.), cultivate în sera Staţiunii Didactice Iaşi. Au fost determinate principalele grupe de microorganisme care colonizează rizosfera şi filosfera acestora. Determinările au vizat numărul total de microorganisme la gramul de sol (în cazul rizosferei) şi la unitatea de suprafaţă (în cazul filosferei - cm^2),bacteriile gram pozitive, bacteriile gram negative şi ciupercile. În plus, pentru o mai bună evidenţiere a diferenţelor care există între cele două specii de plante ornamentale perene la nivel microbiologic, iar în cadrul aceleaşi specii între rizosferă şi filosferă, în cazul ciupercilor au fost determinate şi genurile acestora.*

In the rhizosphere area is present a very high number of microorganisms, which appertain to different taxonomic groups, and their density decrease with the increasing of distance from roots area. In greenhouse soil we have studied the rhizosphere microbiological composition of two ornamental plant species (thuja and boxwood) as structure and rate of participation.

The leaves are populated with different type of microorganism (bacteria, fungus, yeasts, and seaweeds). Natural medium represented by the leaves surface is exposed directly to sun light, climate and temperature variations, humidity and dryness, and also factors related with leaves age. The habitat adjacent to leave is called phyllosphere (gr. „phyllon" – leave) in analogy to the rhizosphere, and the environment direct associated with the leaves, is called phyloplan. The microorganism dispersion from phyllosphere is supported by rains, draught, dust and insects.

In glasshouse, the soil and environment conditions are different in compares to the natural environments, and these differences can produce modifications of the microbiological composition. The aim of this study was to present the results regarding the microbial variety from the rhizosphere and phyllosphere of two ornamental plants cultivated in glasshouse conditions.

MATERIAL AND METHOD

The material necessary for microbiological analysis regarding the rhizosphere and phyllosphere area for the perennial ornamental plants of thuja and boxwood was harvest from the greenhouse belonging to "V. Adamachi" Didactic Farm in January 2008.

For the study of the rhizospheric microflora, the method of Petri dishes culture was used. The plants selected aleatory were harvest entirely, fact that permitted to take the roots with a quantity of soil corresponding to the three zone of rhizosphere. The samples were put in sterile bags for transportation to laboratory. In the same day the external and periradicular soil from plants rhizosphera was discarded and the soil from the internal area was collected on a sterile paper and used for future analysis. After this moment, the soil dilutions were prepared using the successive dilutions method, and the medium from the Petri dishes was contaminated using the inclusion method.

For the study of the phyllospheric microflora leaves from middle of plants, avoiding youngest or older leaves, were harvested. Also, a very important fact is that the leaves must be healthy and characteristic for each plant species. After harvesting, the leaves were putted into sterile bags for transportation to laboratory. There, the leaves were placed on medium culture and imprinted for a short period of time.

For an easy identification of different groups of microorganisms, a different specific medium culture was used. Thus, to determine the total number of microorganism/g soil we used the PDA medium (potato-dextrose-agar), for identification of gram positive bacteria the PDA medium with streptomycin (35 ppm) and for determination of micromycetes number PDA medium with Bengal rose (33 ppm). The inoculation was made, introducing 1 ml of soil dilutions in each Petri dish with molten and cooled medium at 45°C. The inseminated Petri dishes and that with the leavesprint are incubated at 37°C for bacteria and 28°C for fungus. The bacteria colony number was counted after 24 hours, and the fungus colony after 5 days.

RESULTS AND DISCUSSIONS

Rhizosphere

According to the descriptions of different types of symbiosis, they substitute some deficiencies of respective partners and environment, providing an interchange of nutrients, an essential interaction mechanism for the most of symbionts; devices of recognition, defense and plundering aid; protection; ecologic advantages.

Due to these influences, in natural environments, host organisms for the symbionts have a physiology and behavior different from that of axenic organisms, induced by the presence of the symbionts. Symbionts work frequently as autonomic entity and not as different organisms.

A close examination of the biological activity from the rhizosphere of thuja and boxwood show a great variability of the number of microorganisms that carry out their activity in the rhizosphere zone (Table1).

Table 1

Biological activity at rhizosphere level for thuja and boxwood cultivated in greenhouse conditions

Variant	Total microorganism/g soil	Fungi (%)	G^+ bacteria (%)	G^- bacteria (%)
Martor	127600	3.1	9.9	87
Thuja orientalis L.	316500	25.6	12.4	62.0
Buxus sempervirens L.	138450	3.6	5.7	90.7

The results interpretation about the biological activity on thuja (*Thuja orientalis* L.) rhizosphere soils show that the microbial activity is more intense than of boxwood, and even when compared to the witness, represented by an untilled soil specimen.

The total number of microorganisms determined in the rhizosphere of thuja was 316500 for each gram of soil, compared to 127600 determined on control soil sample. The same aspect appears in the case of fungi where the biological activity is a few times bigger than the control sample.

It should be noticed that the relation between microorganisms is completely different; thus, fungi from the rhizosphere of thuja represent 25.6% and the bacteria 74.4%. Among bacteria, the G^- species overcome the G^+ species with 83.3% to 16.7%.

The identification of micromycetes in the rhizosphere area of thuja shrubs shows a relative small number of genera: *Rhizopus, Penicillium, Mucor, Fusarium* and *Aspergillus*. However, the ratio between these groups is very different, with *Penicillium* spp. as dominant genus with 93.6%. The other genera *Rhizopus, Mucor, Fusarium* and *Aspergillus* cumulate together only 6.4% from all fungi (Figure 1).

At boxwood (*Buxus sempervirens* L.) the synthesis of results shows a microbial activity less intense than that of thuja, but greater than that of the control soil sample. Thus, the total number of microorganisms determined in the rhizosphere area was 138450 for one gram of soil compared with 127600 founded in case of control sample.

The best represented microorganisms in the rhizosphere of boxwood shrubs are the G^- bacteria, which represent 90.7% from total and 94.1% from all bacteria. Compared to bacteria (98.2%), the fungi represent a small percent (1.8%) of the microbial activity of boxwoods.

The identification of fungi genera which activates in the rhizosphere of boxwood shows the same spectrum of genera as for thuja, but compares with thuja (25.6%) the percent of micromycetes is very small (3.6%). There have been determined species from the following genera: *Penicillium, Rhizopus, Mucor, Fusarium* and *Aspergillus*.

The ratio between genera is more equilibrated compared to thuja, with a advantage for *Fusarium* spp. (37.5%) followed by *Rhizopus* spp. (25%), *Penicillium* spp. (12.5%), *Mucor* spp. (12.5%) and *Aspergillus* spp. (12.5%) (Figure 1).

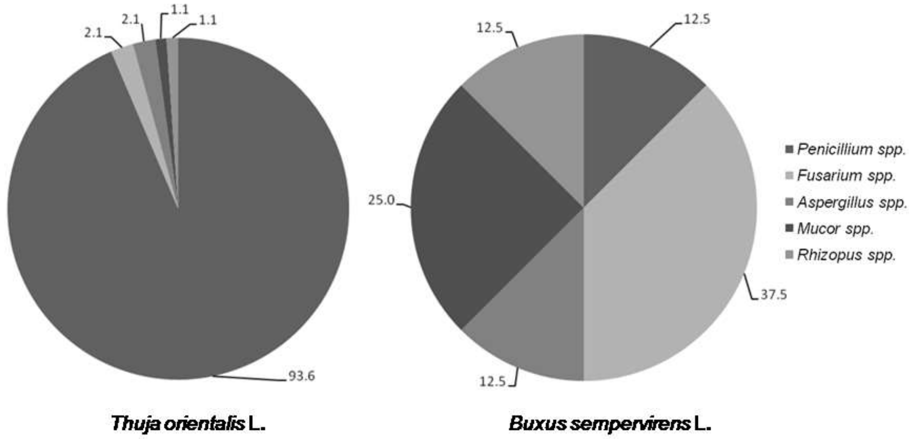

Fig. 1. Distribution of rhizospheric fungi genera for *Thuja orientalis* L. şi *Buxus sempervirens* L.

The existence of the same genera of fungi population, reduced as number, in the rhizospherical area of this two shrubs species can be explained through the long period of their time living in the greenhouse soil, fact that permitted a selection of micromycete genera. The selection was made on basis of biochemical proprieties of the radicular secretion via elimination of the genera that could not use them for their vital processes.

Phyllosphere

The study of microbiological activity from thuja and boxwood phyllosphere cultivated in the glasshouse of "V. Adamachi" Didactical Station Iasi, with the determination of the number of colonies of microorganisms/cm^2 leaf, shows differences between the species, but this are correlated with literature data.

On thuja (*Thuja orientalis* L.) phyllosphere a total number of 5.7 colonies/cm^2 were counted, and from this total 3.8 were micromycete, 0.1 G^+ bacteria and 1.8 G^- bacteria colonies (Table 2).

Table 2

Biological activity at phyllosphere level for thuja and boxwood cultivated in greenhouse conditions

Variant	Total microorganism/g soil	Fungi (%)	G^+ bacteria (%)	G^- bacteria (%)
Thuja orientalis L.	5.7	66.7	1.8	31.6
Buxus sempervirens L.	7.8	79.5	1.3	

The analysis of the isolated micromycete from the surface of thuja leaves show a dominancy of *Cladosporium* genera with 62.1 % from total, follow at a great distance by: *Fusarium* spp. (12.3%), *Penicillium* spp. (10 %), *Alternaria* spp. (7.7 %), *Botrytis* spp. (7.0%) and *Rhizopus* spp. (0.4 %).

The presented genera, excepting *Botrytis* spp., are characterized by a strong antagonism against the colonising species, fact which will explain why these shrubs have a strong resistance at diseases in comparison with other species. That means that phyllosphere plays an important role in plant's life, influenced their health.

Surprising was the small number of bacteria colonies with only 1.9 microorganisms/cm^2, knowing the fact that the greenhouse glass stops the UV radiations. We expected a bigger number, but the results shows us that the symbioses is the results of a long time evolution and the influence of the environmental factors do not cancel, even for a short time, the relationship established between the plants and the microorganism.

At boxwood (*Buxus sempervirens* L.) situation is quite the same as at thuja, a number of 7.8 colonies/cm^2 were isolated, from which 6.2 are micromycete, 0,1 are G^+ bacteria and 1.5 are G^- bacteria.

Regarding the micromycete diversity on boxwood leaves the *Cladosporium* genera represent 72.5% from the total. In boxwood phyllosphere, compares to thuja, new genus of fungi were found: *Aspergilllus* 1.7%, *Verticillium* 2.3%, *Melanospora* 0.6%, *Trichoderma* 0.9%, two genus have been disappeared (*Rhizopus*, *Botrytis*) and the participation rate of *Penicillium* (1.1%) şi *Fusarium* (4.3%) was reduced (Figure 2).

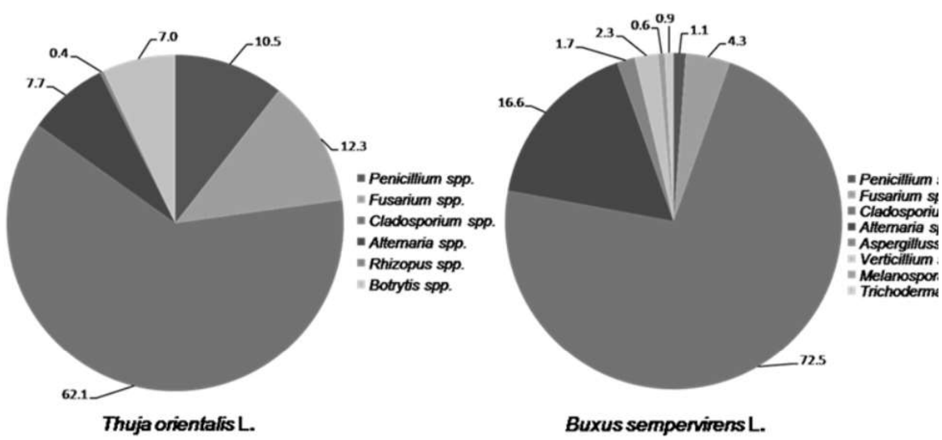

Fig. 2. Distribution of phyllospheric fungi genera for *Thuja orientalis* L. şi *Buxus sempervirens* **L**

CONCLUSIONS

The observations of the microbiological activity at rhizospheric and phyllospheric area on thuja and boxwood shrubs cultivated in glasshouse lead us to the following conclusions:

1. Microbial activity on rhizosphere area for the analyzed species is variable; the rate of participation for the principal groups is characteristic to each species.

2. At rhizospheric level bacteria are the dominant species from the microbial genera, the micromycete representing only a small percentage.

3. At phyllospheric level were isolated species of *Trichoderma* genus, known as antagonists, which maintain the pathogen species in the glasshouse environment under control.

4. The non-cultivated soil has a lower biological activity compare to the rhizosphere, regarding the total number of microorganism and the micromycete diversity.

5. The isolated micromycete from the analyzed shrubs rhizosphere are identical in the same environment conditions; the difference between the thuja and boxwood is on the participation rate and number of micromycete/g soil.

6. From all micromycete isolated in the phyllosphere of both species *Cladosporium* spp. dominate with over 60% from all genera, followed by: *Fusarium* spp., *Penicillium* spp., *Alternaria* spp., *Aspergilus* spp., *Verticillium* spp., *Melanospora* spp., *Trichoderma* spp., *Rhizopus* spp.

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