DIVERSITY OF MICROBIAL COMMUNITIES IN THE PHYLLOSPHERE OF ORNAMENTAL PLANTS

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Abstract

Researches were carried out on three species of ornamental plants, *Chrysanthemum indicum* L. (commonly known as Indian chrysanthemum), *Alstroemeria aurantiaca* L. (commonly known as Peruvian lily or lily of the Incas) and *Pelargonium peltatum* L. (common names include ivy-leaf geranium or cascading geranium) cultivated in the greenhouse and also, on the experimental fields of USAMV Iasi. Anatomical and physiological characters of leaf surface and their physic-chemical environments substantially influence the density and diversity of phyllosphere-inhabiting microorganisms, which may include natural antagonists of important pathogens. The main objectives of this investigation were to study the phyllosphere microbial diversity from both environment (inside and outside) and to quantify the phyllosphere (i.e. leaf surface) microbial population from the ornamental plants. Also, fungal genera were identified for a better understanding of the influence of UV radiation, fluctuating temperature, nutrient resources and relative humidity on fungal diversity from both, inside and outside cultivated species.

Key words: phyllosphere, microbiota, ornamental plants

The microbial communities of leaves are represented by a variety of bacteria, filamentous fungi yeasts, algae, and, less frequently, protozoa and nematodes. The habitat adjacent to leaves is called phyllosphere (gr. „phyllon" – leave) and the inhabitants are called epiphytes. The environment direct associated with leaves is called phyloplan. (Lipsa et al, 2008) The structure of microbial phyllosphere communities is influenced by numerous environmental parameters including UV radiation, air pollution, relative humidity, nutrients and temperature.

The phyllosphere is an open system and microbes can invade plant leaves by migration from the atmosphere, soil, other plants, insects, and animals. The microbial populations from the aerial habitat of plants (phyllosphere) are involved in functional processes as large in scale as the carbon cycle, nitrogen fixation, and degradation of organic pollutants, pesticide residues.

The global population of phyllosphere microbes is estimated to be approximately 10^{26} cells. Bacteria are by far the most abundant inhabitants of the phyllosphere, often being found in numbers around 10^6 to 10^8 cells/cm^2 (Leveau 2006). Filamentous fungi are considered transient inhabitants of leaf surfaces, being present predominantly as spores.

In this study we describe the diversity of phyllosphere microbial communities on three ornamental plants cultivated in two different environments: a greenhouse and an experimental field.

MATERIAL AND METHOD

The biological material (leaves) necessary for microbiological analysis regarding the phyllosphere from the perennial ornamental plants *Chrysanthemum indicum* L., *Alstroemeria aurantiaca* L. and *Pelargonium peltatum* L. was harvest from a greenhouse and an experimental field belonging to "USAMV Iasi in 2014.

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 nutritive media and imprinted for a short period of time.

The experiment was conducted with a threefold repetition for each microbiological determination and the counts obtained were averaged. Microbiological media plates were prepared using Masterclave 09 plate maker and an aliquot portion of 15mL of media was poured using APS 320 automated Petri plate filler (AES Laboratoire, France).

For an easy identification of different groups of microorganisms, different nutritive media were used. Thus, to determine the total number of

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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 imprinted Petri dishes and that with the leavesprint are incubated at 37℃ for bacteria and 28℃ for fungus. The bacteria colony number was counted after 24 hours, and the fungal colony after 5 days.

Light microscopy (1000x magnification) was used to determine the colonial features and the morphological structures of the fungi. The determination of the morphological structures of fungi was carried out on fungal material mounted in lactophenol by slide culture technique. Fungi were identified to genus level based on morphological and physiological characteristics following the works provided by Ellis (1971,1997), De Hoog et al. (2000), Barnett and Hunter (1999).

RESULTS AND DISCUSSION

The study of microbiological activity from the phyllosphere of the perennial ornamental plants *Chrysanthemum indicum*, *Alstroemeria aurantiaca* and *Pelargonium peltatum* cultivated in a greenhouse and an experimental field started with the determination of the number of colonies of microorganisms/cm^2 leaf and shows differences between the species and cultivation environment, respectively.

Between the cultivation environments the obtained results representing the number of microorganisms reported to cm^{-2} for the same ornamental plant species were no statistically significant. For example, on Indian chrysanthemum (*Chrysanthemum indicum* L.) leaf surface a total number of 7.6 CFU/cm^2 were counted in case of greenhouse environment, and 7.5 CFU/cm^2 in case of field environment. Between the principal groups of microorganisms the differences between environments were important only in case of Gram negative bacteria, 1.1 CFU/cm^2 for greenhouse and 0.4 CFU/cm^2 for outside field (Table 1).

Surprising small was the number of bacteria colonies, knowing the fact that the greenhouse glass stops the UV radiations. We expected a higher 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.

This tendency could be observed in case of all three ornamental plants cultivated outside the greenhouse and in case of almost all Gram positive and - negative bacteria.

The reducing of Gram negative bacteria population in outside environment could be explained by high levels of UV exposure, leaves age and morphology, water stress, fluctuating temperature and heterogeneous nutrient availability (Maignien et al, 2014).

Table 1

Biological activity at phyllosphere level for ornamental plants cultivated in different environments

Ornamental plant	Greenhouse environment				Outside environment			
	Microbiota (CFU/cm^2)	Micromycetes (CFU/cm^2)	G- bacteria (CFU/cm^2)	G+ bacteria (CFU/cm^2)	Microbiota (CFU/cm^2)	Micromycetes (CFU/cm^2)	G- bacteria (CFU/cm^2)	G+ bacteria (CFU/cm^2)
Chrysanthemum indicum	7.6	4.3	1.1	2.2	7.5	4.4	0.4	2.7
Alstroemeria aurantiaca	6.1	2.1	1.5	2.5	5.0	1.9	0.7	2.4
Pelargonium peltatum	8.2	0.5	1.1	6.6	7.9	1.0	1.3	5.6

The analysis of the isolated filamentous fungi from the surface of Indian chrysanthemum (*Chrysanthemum indicum* L.) leaves show a dominancy of *Aspergillus* genera with 67.1% from total in case of greenhouse environment and *Penicillium* genera with 43.6% from total in case of outside environment. In case of the protected environment the total number of genera was six, and *Aspergillus* genera was followed at a great distance by: *Penicillium* spp. (15.3%), *Fusarium* spp. (8.2%), *Rhizopus* spp. (4.7%), *Trichoderma* spp. (3.5%) and *Alternaria* spp. (1.2%).

In case of the external medium, on the phyllospehere area were found ten fungal genera. The highest concentration was found in case of *Penicillium* spp followed by: *Aspergillus* spp. (18.6%), *Rhizopus* spp. (18.2 %), *Micellia sterilia* (7.1%), *Trichoderma* spp. (5.1%), *Fusarium* spp. (4.4%), A*lternaria* spp. (0.7%), *Verticillium* spp. (0.7%), *Sepedonicus* spp. (0.7%) and *Nigrospora* spp. (0.3%).

The presented genera 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.

Differences between both environments could be explained by the existence of a higher

concentration of fungal spores in the outside air. (*figure 1*).

Regarding the micromycete diversity on Peruvian lily (*Alstroemeria aurantiaca* L.) leaves, we observed almost the same situatios as in case of *Chrysanthemum* spp. The number of genera was higher in the exterior environment (6) in comparacy to the glasshouse environment (4). *Aspergillus* genera represent 53.7% from the total micromycetes isolated in the protected environment, while *Rhizopus* spp is on the first position in the exterior with 49.1%. In the lily phyllosphere grown in glasshouse compares to field Rhizopus genera was absent (*figure 2*).

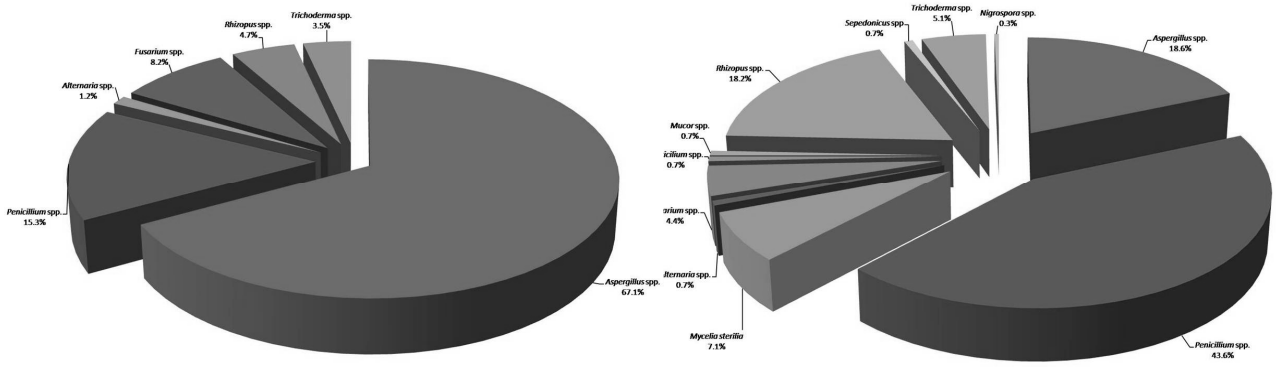

Figure 1 **Frequency of isolated fungi from the *Chrysanthemum indicum* L. leaves cultivated in greenhouse (left) and exterior (right) environments**

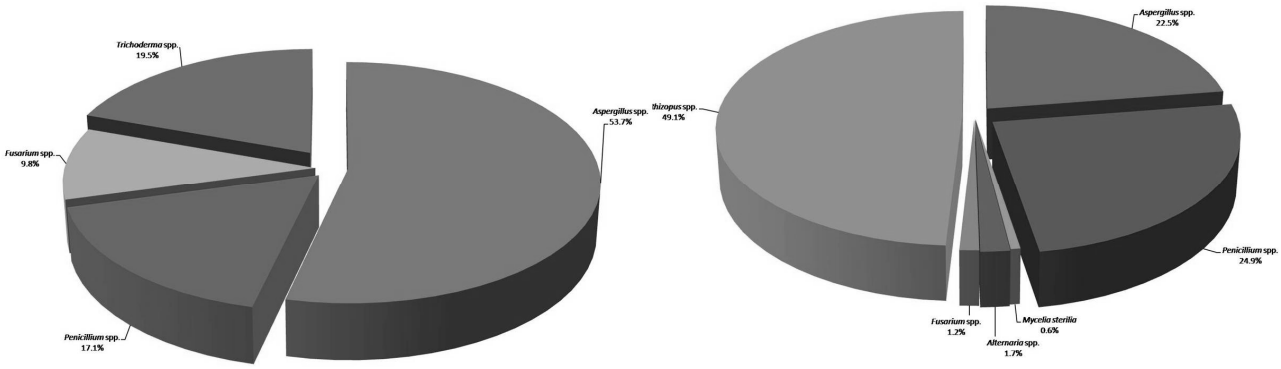

Figure 2 **Frequency of isolated fungi from the *Alstroemeria aurantiaca* L. leaves cultivated in greenhouse (left) and exterior (right) environments**

Fungal diversity on cascading geranium (*Pelargonium peltatum* L.) leaves in greenhouse environment is represented by four genera: *Aspergillus* (90.1%), *Rhizopus* (4.2%), *Trichoderma* (3.1%) and *Penicillium* (2.6%). In cascading geranium phyllosphere cultivated on field six genera of fungi were isolated: *Trichoderma* (33.3%), *Penicillium* (2.6%), *Aspergilllus* (15.3%), *Fusarium* (907%), *Rhizopus* (5.6%) and *Alternaria* (5.6%) (*figure 3*).

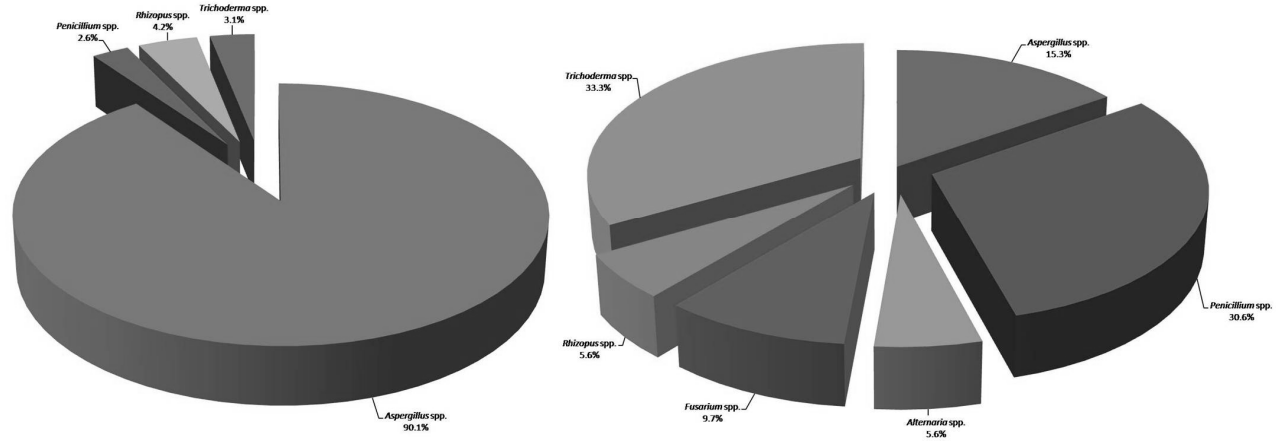

Figure 3 **Frequency of isolated fungi from the *Pelargonium peltatum* L. leaves cultivated in greenhouse (left) and exterior (right) environments**

CONCLUSIONS

The observations of the microbiological activity at phyllospheric area on three different ornamental plants cultivated in protected- and field environments lead us to the following conclusions:

The phyllosphere of each ornamental plant was a unique habitat and contained diverse microorganisms with a variable activity; the rate of participation for the principal groups is characteristic to each plant species.

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

Between the cultivation environments the obtained results representing the number of microorganisms for the same ornamental plant species were no statistically significant.

The maximal number of fungal genera was found for all plant species in case of the field environment: *Chrysanthemum indicum* L. (10 genera), *Alstroemeria aurantiaca* L. (6 genera) and *Pelargonium peltatum* L. (6 genera).

The dominant genera isolated from *Chrysanthemum indicum* L. was *Aspergillus* with 67.1%, in case of *Alstroemeria aurantiaca* L. was *Rhizopus* spp with 49.1% and for *Pelargonium peltatum* L. was *Trichoderma* with 33.3%, respectively.

The number of genera isolated from the plants cultivated in greenhouse varied from four (*Alstroemeria* spp. and *Pelargonium* spp.) to six (*Chrysanthemum* spp.), but for all plant species *Aspergillus* spp. was the dominant genera.

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

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