PHYLLOSPHERE MICROBIAL PROFILE OF ORNAMENTAL PLANTS GROWN IN INDOOR AND OUTDOOR ENVIRONMENTS

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Abstract

The aerial surfaces of plants, or phyllosphere, represent unique and wide habitats for microbial communities, which play a key role in plant growth and adaptation to adverse conditions. The phyllosphere microbiota is composed mainly of bacteria and fungi, and less frequently are present viruses, cyanobacteria, and protozoans. Leaf-inhabiting fungi and bacteria are important, but often overlooked component of biodiversity studies. To understand their diversity and function in relation to plant species and climate, the phyllospheres of five phylogenetically diverse ornamental plant species (*Vinca minor*, *Aster dumosus*, *Chrysanthemum indicum*, *Stachys lanata*, and *Sedum spectabile*) were analyzed under indoor and outdoor conditions. For both environment, *Stachys lanata* showed the lowest absolute abundance and diversity of fungi and bacteria, while *Aster dumosus* had the highest abundance and the diversity of fungi in comparacy to the other four plant species. Fungal isolates were abundantly ascomycetes and predominated by commonly known endophytic genera, such as *Penicillium*, *Aspergillus*, *Alternaria*, *Rhizopus*, and *Fusarium*. In the outdoor environment was a smaller proportion of shared fungal genera among the five different plants, in comparacy to indoor controlled greenhouse. For a better knowledge of the impact of plant species, UV radiation, variable temperature, nutritional resources, and relative humidity on microbial biodiversity, fungal genera from inside and outside cultivated species were identified.

Key words: phyllosphere, ornamental plants, microbial communities

The phyllosphere microbiota comprises all microorganisms living on the aerial or above-ground plant parts, including leaves, stems, flowers, and fruits, with leaves as the most dominant part. The phyllosphere microbiome is composed of bacteria, filamentous fungi, yeasts, algae, viruses and, less frequently, protozoa and nematodes (Bashir I. *et al*, 2021; Lemanceau P. *et al*, 2017). The leaf surface is a large and extremely diverse habitat for various microbial organisms, including bacteria and fungi, and estimated to exceed 10^8 km^2 and around 60% of the biomass across all taxa on Earth (Bao L. *et al*, 2019; Koskella B, 2020). Moreover, it is estimated that phyllosphere bacterial abundance may exceed 10^{62} cells globally, and it is much higher than that of fungi (Jia T. *et al*, 2020). The leaves colonization by microorganisms starts at seedling emergence and, in the case of perennial plant species, it starts again every year at bud burst by means of horizontal propagation from various environmental sources (Jia T. *et al*, 2020; Lemanceau P. *et al*, 2017). 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 are involved in functional processes as large in scale as the carbon cycle, nitrogen fixation, and degradation of organic pollutants, pesticide residues (Lipsa *et al*, 2015).

The microbial inhabitants structure of this heterogeneous habitat is influenced by host plant and numerous environmental parameters including ultraviolet radiation, air pollution, relative humidity, nutrients availability and temperature fluctuations (Vorholt J.A., 2012). In case of leaf-associated fungal communities, Mahnert A. *et al* (2018) identified for instance land use, host genetics, tissue age or host defense compounds as important factors which play a role in the fungal diversity of phyllosphere.

To study the variability of the microbiome of indoor and outdoor plants in relation to plant species and environment we selected different ornamental plant species grown in the greenhouses and on experimental fields from Iasi University of Life Sciences.

MATERIAL AND METHOD

The biological material (leaves) necessary for microbiological analysis regarding the

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phyllosphere from the perennial ornamental plants *Vinca minor*, *Aster dumosus*, *Chrysanthemum indicum*, *Stachys lanata*, and *Sedum spectabile* was harvest from a greenhouse and an experimental field belonging to USV Iasi in 2021.

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 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 28°C. 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 DISCUSSIONS

The study of microbiological activity from the aerial surfaces of ornamental plants *Vinca minor*, *Aster dumosus*, *Chrysanthemum indicum*, *Stachys lanata*, and *Sedum spectabile* cultivated in indoor (greenhouse) and outdoor (experimental field) environment started with CFU (colony-forming unit)/cm^2 leaf determination and shows differences between the plant 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 statistically significant ($p<0.05$) only in case of *Aster dumosus* and *Chrysanthemum indicum*. For example, on Indian chrysanthemum (*Chrysanthemum indicum* L.) leaf surface a total number of 39.9 CFU/cm^2 were counted in case of indoor environment, and 31.3 CFU/cm^2 in case of outdoor environment. Between the principal microbiota groups the differences between environments were important only in case of Gram negative bacteria, 12.5 CFU/cm^2 for greenhouse and 3.7 CFU/cm^2 for outside field (*table 1*).

The quantity of bacteria colonies was surprisingly low, given that the greenhouse glass blocks UV rays. We expected a bigger number, but the data suggest that symbioses are the consequence of long-term evolution, and that the influence of environmental conditions does not erase the association created between plants and microorganisms, even if just for a short period.

This trend could be seen in practically all Gram positive and negative bacteria, as well as all ornamental plants species grown outside the greenhouse.

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)	Fungi (CFU/cm^2)	G- bacteria (CFU/cm^2)	G+ bacteria (CFU/cm^2)	Microbiota (CFU/cm^2)	Fungi (CFU/cm^2)	G- bacteria (CFU/cm^2)	G+ bacteria (CFU/cm^2)
Vinca minor	*17.4*	9.6	5.1	2.7	*15.9*	7.3	4.7	3.9
Aster dumosus	*34.3*	24.7	8.1	1.5	*28.6*	15.9	6.3	6.4
Chrysanthemum indicum	*39.9*	21.6	12.5	5.8	*31.3*	23.3	3.7	4.3
Stachys lanata	*3.5*	1.5	0.9	1.1	*3.1*	1.9	0.2	1.0
Sedum spectabile	*12.1*	6.0	2.5	3.6	*8.8*	5.2	2.5	1.1

The analysis of the isolated filamentous fungi from the surface of Lesser periwinkle leaves (*Vinca minor* L.) show a dominancy of *Alternaria* genera with 25.3% from total in case of outdoor environment and *Aspergillus* genera with 30.5% from total in case of inside environment. In case of the protected environment the total number of genera was six, and *Aspergillus* genera was followed by: *Penicillium* spp. (25.4%), *Fusarium* spp. (16.9%), *Alternaria* spp. (15.3%), *Trichoderma* spp. (8.5%) and *Rhizopus* spp. (3.4%)

In case of the external medium, on the phyllospehere area were found ten fungal genera. The highest concentration was found in case of A*lternaria* spp. followed by: *Aspergillus* spp. (16.9%), *Penicillium* spp (14.0%), *Fusarium* spp. (12.9%), *Trichoderma* spp. (8.4%), *Rhizopus* spp. (7.9 %), *Micellia sterilia* (5.6%), *Cladosporium* spp. (5.6%), *Mucor* spp. (2.2%), and *Geotrichum* spp. (1.1%).

Differences between both environments could be explained by the existence of a higher concentration of fungal spores in the outside air. (*figure 1*)

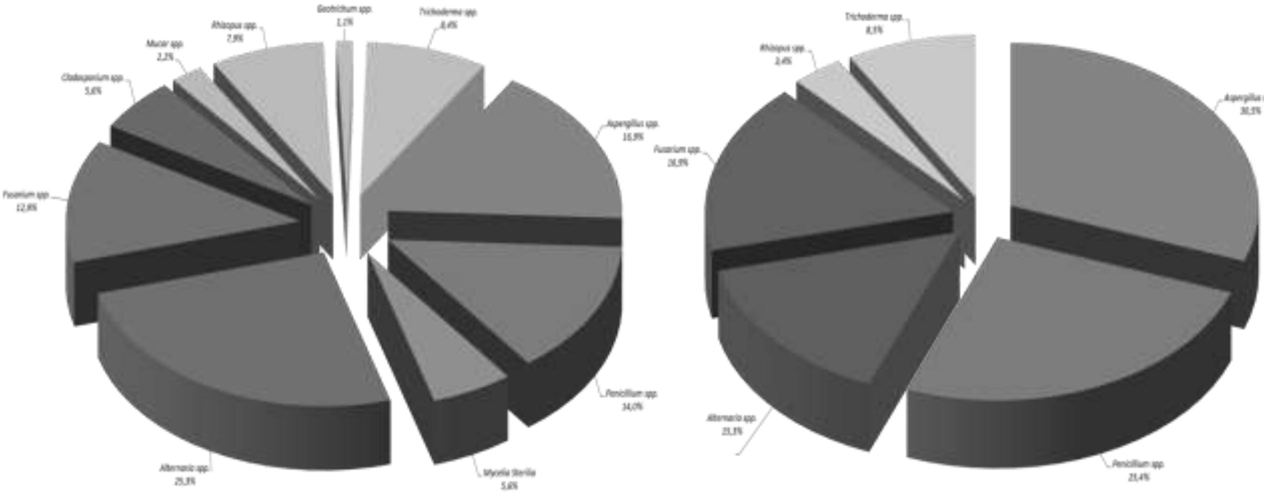

Figure 1 **Frequency of isolated fungi from the *Vinca minor* leaves cultivated in indoor (right) and outdoor (left) environments**

Regarding the micromycete diversity on Rice button aster leaves (*Aster dumosus* L.), almost the same situations as in case of *Vinca* sp was observed. The number of genera was higher in the outsider environment (8) in comparacy to the glasshouse environment (5). *Alternaria* genera was the dominant genera in both environments, and represent 43.8% from the total micromycetes isolated in the exterior environment, and 79.6% in the indoor. In the aster phyllosphere grown in glasshouse compares to field *Cladosporium*, *Mucor*, *Mycela sterilla* and *Rhizopus* genera were absent (*figure 2*).

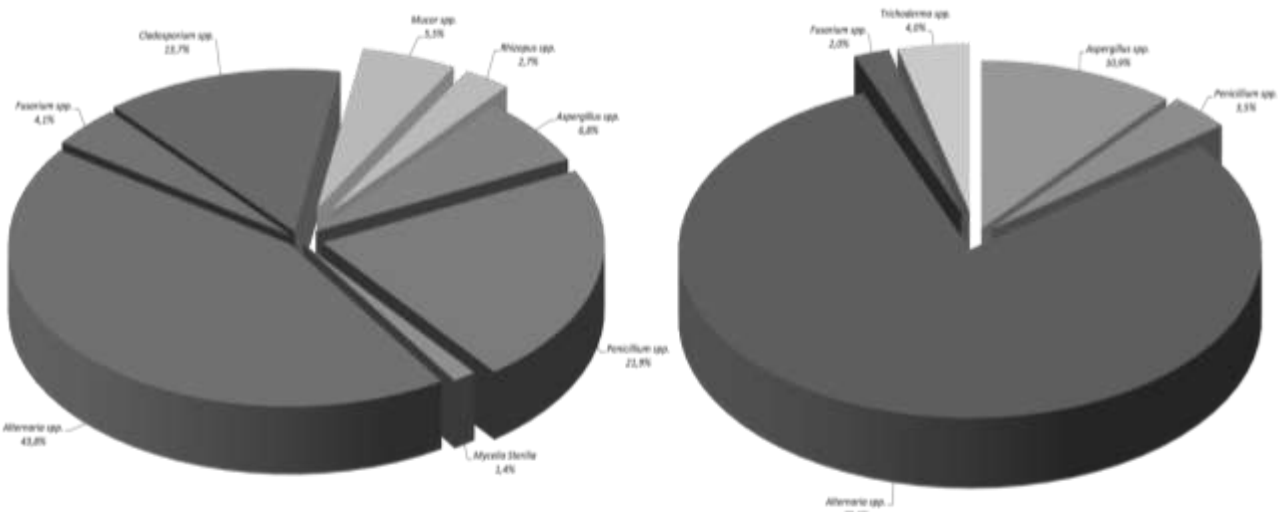

Figure 2 **Frequency of isolated fungi from the *Aster dumosus* leaves cultivated in indoor (right) and outdoor (left) environments**

Fungal diversity on Indian chrysanthemum cascading geranium leaves (*Chrysanthemum indicum* L.) in external environment is represented by seven genera: *Alternaria* (40.4%), *Penicillium* (21.6%), *Mycela sterilla* (14%), *Trichotecium* (11.7%), *Aspergillus* (5.8%), *Fusarium* (4.1%), and *Rhizopus* (2.3%), In iris phyllosphere cultivated in greenhouse five genera of fungi were isolated: *Aspergilllus* (70.6%), *Penicillium* (11.9%), *Alternaria* (7.9%), *Rhizopus* (5.6%), and *Trichoderma* (4.0%) (*figure 3*).

The given genera have a strong antagonistic relationship with the colonizing species, which helps to explain why these shrubs are more disease resistant in comparison to other species. This means that the phyllosphere has a significant impact on plant life and health.

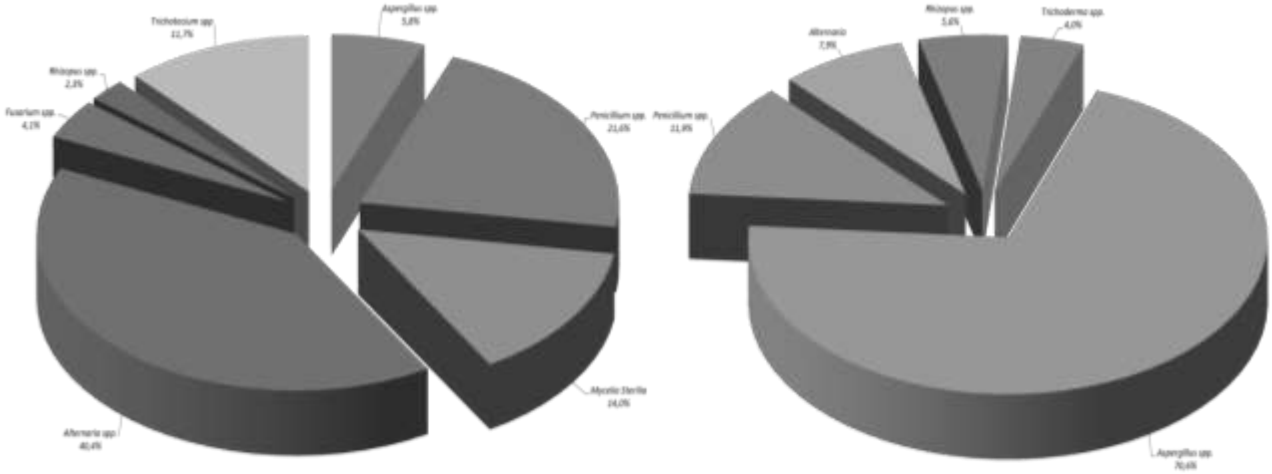

Figure 3 **Frequency of isolated fungi from the *Chrysanthemum indicum* leaves cultivated in indoor (right) and outdoor (left) environments**

Regarding the number and diversity of the filamentous fungi genera isolated from lamb's ear (*Stachys lanata* K. Koch) leaves surfaces, the lowest values observed were noticed. One of the important causes of the registerd results could be explained through the morphological leaves type, soft and fur-like hair coated.

For the first time in this study, the registered number of genera was lower in the outdoor environment (4) in comparacy to the glasshouse environment (6). *Aspergillus* genera represent 30.5% from the total micromycetes isolated in the protected environment, while *Alternaria* spp. is on the first position in the exterior with 65.4% (*figure 4*).

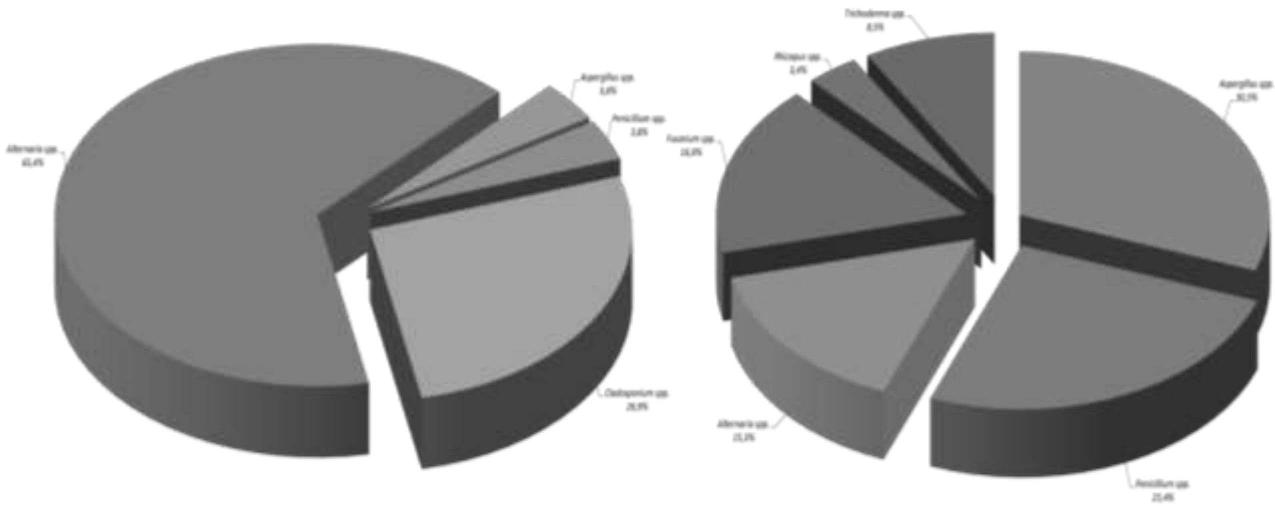

Figure 4 **Frequency of isolated fungi from the *Stachys lanata* leaves cultivated in indoor (right) and outdoor (left) environments**

The analysis of the isolated filamentous fungi from the surface of iceplant leaves (*Sedum spectabile* B.) show a dominancy of *Alternaria* genera with 43.5% from total in case of outdoor environment, and *Aspergillus*, *Alternaria* and *Penicillium* genera with 26.6% each, from total in case of inside environment. In case of the greenhouse environment the total number of genera was seven, *Aspergillus*, *Alternaria* and *Penicillium* spp. genera being followed by

Mycellia sterilia (10.7%), *Fusarium* spp. (5.3%), *Trichoderma* spp. (2.1%) and *Rhizopus* spp. (2.1%)

In case of the external medium, on the phyllospehere area were found five fungal genera. The highest concentration was found in case of *Alternaria* spp. followed by: *Aspergillus* spp. (29.0%), *Penicillium* spp (14.5%), *Fusarium* spp. (7.2%), and *Rhizopus* spp. (5.8 %) (*figure 5*).

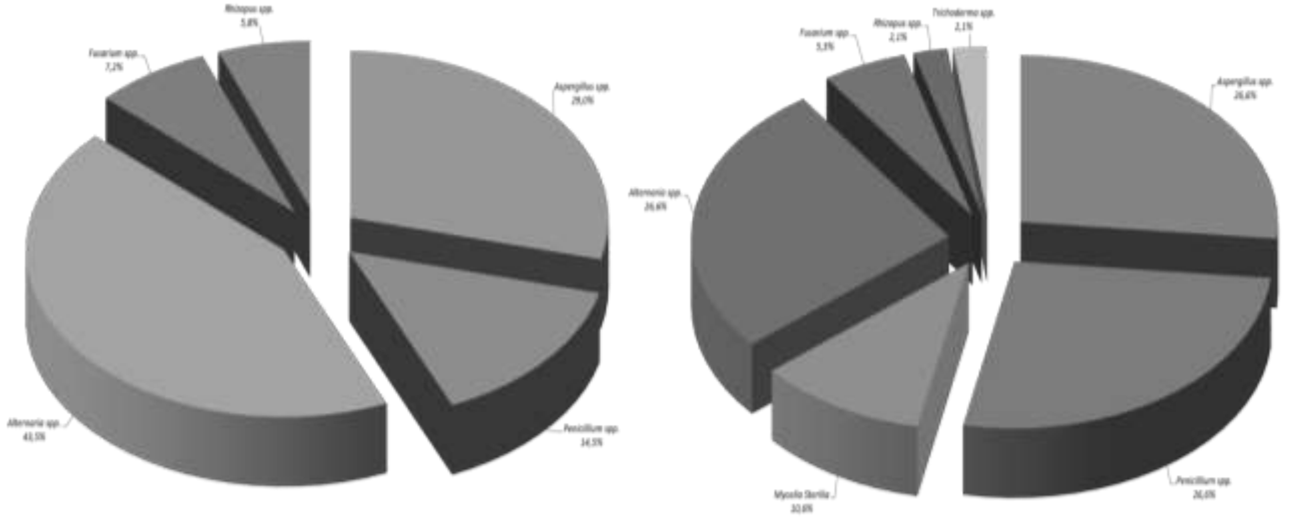

Figure 5 **Frequency of isolated fungi from the** *Sedum spectabile* **leaves cultivated in indoor (right) and outdoor (left) environments**

CONCLUSIONS

This microbiological study about microbial presence at phyllospheric area on five different ornamental plants cultivated in indoor and outdoor environments lead us to the following conclusions:

The abundance, species richness and similarity of the phyllospheric microbial communities among the five ornamental plants demonstrate the role of host plant identity in driving phyllosphere epiphytic and endophytic community structure.

Each ornamental plant's leaf surface represents a distinct environment, with a broad range of microorganisms with varying levels of activity; the rate of participation for the major microbial groups differed by plant species.

Between the cultivation environments the obtained results representing the number of microorganisms for the same ornamental plant species were statistically significant only in case of *Chrysanthemum indicum* L.

The maximal number of fungal genera was found for *Vinca minor* (10 genera), *Aster dumosus* (8 genera) and *Chrysanthemum indicum* (10 genera) in case of the exterior environment and *Sedum spectabile* (7 genera) and *Stachys lanata* (6 genera) in greenhouse.

For the outdoor environment the dominant genus isolated from all five ornamental plant species was *Alternaria* with values from 25.3%, in case of *Vinca minor* L. to 65.4% in case of *Stachys lanata*. In case of indoor space, the dominant genera were *Alternaria* (79.6%) registered for *Aster dumosus* and *Aspergillus* (70.6%) for *Chrysanthemum indicum*

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

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