DIVERSITY OF CULTIVATED MICROSCOPIC FUNGI IN VIETNAM FORESTS AND THEIR ROLE IN THE DECOMPOSITION OF THE LEAF LITTER

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1. INTRODUCTION

Fungi are the one of the key components of ecosystems [6, 7]. Despite the fact that they are always present in all habitats on any substrates, it is not always possible to observe them directly in nature. This is due to their relatively poor knowledge, especially in tropical forest ecosystems.

The forests of the tropics are characterized by a high species diversity of fungi, many of which are still unknown to science [4, 11, 12, 13, 14]. The structural organization and functional activity of their complexes are also insufficiently investigated [19].

Usually microscopic fungi are completely invisible due to their small size. They constantly grow on all substrates, they are most abundant in the upper horizon of the soil and leaf litter, phytopathogenic species are present on living plants, entomopathogenic species grow on insects. Micromycetes contribute to the processes of soil formation and regulation of species composition and physiological activity of soil biota and terrestrial vegetation [6, 16]. Sample selection and laboratory researches are necessary for their study.

Works with tropical fungi have practical importance. Micromycetes can be used as producers of biologically active substances (antibiotics, growth regulators, toxins, enzymes, etc.) [5].

The objective of this research was to study the structure of the complexes and the functional activity of microscopic fungi in soils and plant litter of various biotopes of specially protected forests in Vietnam.

2. MATERIALS AND METHODS

Systematic studies of species diversity, distribution patterns, substrate development and ecology of micromycetes from Vietnamese soils have been carried out since 2009 on the basis of the Joint Russian-Vietnamese Tropical Research and Technology Center [1, 15].

The analysis of the collected material was carried out by inoculation from serial dilutions of Z. Waksman on the solid nutrient media in the modification of D.G. Zvyagintsev [22]. There were two culture media in our research, that allow to reveal a wide range of micromycetes and provide easily differentiate the morphological types of colonies in samples: Czapek agar with 0.3% of sucrose and malt extract agar. An antibiotic was added to suppress the growth of bacteria in the medium.

Identification with using morphological features was performed according to generally accepted determinants and articles containing the research of individual genera and species description [8, 10, 20]. The names of species and systematic position were given on databases: The MycoBank Fungal databases and CABI Bioscience Databases.

The obtained cultures of micromycetes are stored in the collection of the Department of mycology and algology of the Biological faculty of Moscow State University. The representation of the species was estimated from the spatial frequency of occurrence and relative abundance [18, 21].

Representation of species was evaluated by indicators of spatial frequency of occurrence and relative abundance [18, 21]. The concept of a complex of typical species, isolated on the basis of frequency of occurrence, was used [18] to characterize the species composition of soil micromycetes. Estimation of species diversity was done with using information indexes that describe two main aspects of diversity: richness and complexity [17].

Visualization of the degree of similarity of the species composition of the micromycetes of the studied samples was carried out by ordination method [9]. According to purpose the analysis was carried out by the principal component method in the PCO3 program [2].

3. RESULTS AND DISCUSSION

As a result of research on the territory of Vietnam's 13 specially protected natural areas (SPAs), 492 species from 136 genera of microscopic fungi was found in soil and associated substrates (plant residues from soil, "air" soil and litter, decaying wood, excrement of worms, etc.).

Despite years of research and data accumulation, new species are identified each year (table 1), even if the studies were repeated in places where work was done in previous years. This fact indicates a great variety of microscopic fungi of Vietnam and the necessity of long standing studies of each territory to identify the total diversity and evaluate their role in the decomposition of organic plant residues and potential as producers of biologically active substances.

It is possible to conduct a preliminary comparative analysis of the diversity of soil micromycetes of various territories in Vietnam that based on the accumulated data. Differences in the species composition are clearly demonstrated in the ordination diagram of the complexes of micromycetes of the studied habitats and substrates by the main component method (fig. 1).

Table 1. The number of species of soil micromycetes found during the work in the specially protected territories of Vietnam from 2009 to 2016

Period of work	Number of identified species	Number of species not previously found	Total number of isolated species
2009 december	104	104	104
2011 july	185	148	252
2012 april	120	51	303
2012 (2) december	144	52	355
2013 april	98	20	375
2013 may	88	16	391
2014 may-june	193	47	438
2015 march-april	124	24	461
2016 may-june	155	30	492

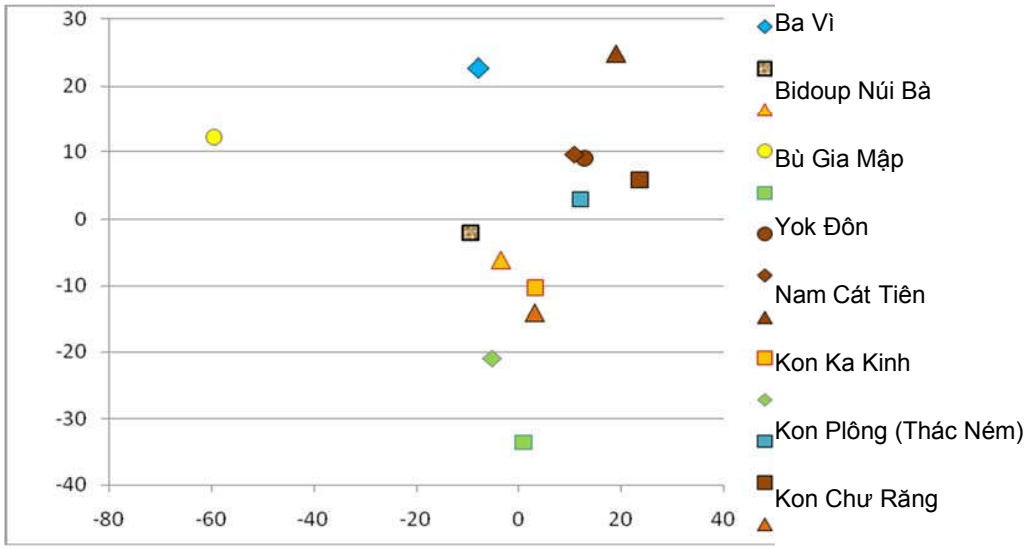

Figure 1. Ordination of the identified species composition of soil microscopic fungi in the SPAs of Vietnam (13 national parks and reserves), researched for the period from 2009 to 2016. The main component method using the Brey-Curtis distinction in the PCO3 program [2], based on only the presence/absence of species

During the analysis of the summary lists of species identified for all SPAs (13 national parks and reserves) (fig. 1), it is clear that the forests are divided into dry (open dipterocarp forests of the Yok Don National park) and more humid forests (all others) along the horizontal axis, which describes 19% of variation. The second axis, describing 12% of variation, divides places more or less by geographical location.

The lowland forests of southern Vietnam (Cát Tiên National park, Đồng Nai Biosphere reserve and Đồng Nai Culture and Nature reserve, Đồng Nai Biosphere reserve) are in lower part of diagram. The group, which includes the mountain and piedmont forests of the southern tip of the Tay Nguyen plateau and adjacent hilly territories (Lộc Bắc forest enterprise, Bù Gia Mập National park, Bidoup - Núi Bà, Chư Yang Sin National park) is the following. It is interesting to notice that the mountain forests of Bidoup - Núi Bà National park and Chư Yang Sin National park differ from each other more than from piedmont forests on the hills, although they are adjacent to each other on the Dalat plateau, probably because they are located on different sides of the massif and they are affected by the related climatic characteristics.

The next group includes mountain forests in the central part of the plateau of Tay Nguyen (Kon Ka Kinh National park, Kon Plông Protected forest (Thác Ném), Chư Mom Ray National park) and the northern karst massif (Xuân Sơn National park).

The natural reserve of Kon Chư Răng Nature reserve out of this group. This can be explained by less human activity. It contains native tropical forests not affected by the logging, and generally the anthropogenic influence is much lower than in other research locations.

The separate position of the Ba Vì National park is difficult to discuss, it can be explained by an insufficient amount of processed material from this location.

As a result of the work, the features of the structure of the species composition of soil micromycetes of tropical forests in comparison with these fungi of temperate forests were first revealed. It was found: 1) generally higher species and taxonomic diversity of studied micromycetes; 2) low species richness and number of representatives of the department of Zygomycota; 3) a significant proportion of micromycetes from the order Hypocreales; 4) the noticeable participation of fungi of the order Xylariales in the general species richness of soil micromycetes; 5) high diversity of representatives of the genus *Aspergillus* and related teleomorphs; 6) a relatively large number of micromycetes, known as potential pathogens of plant diseases; 7) a great variety and abundance of morphological forms of fungi that do not form sporulation on universal nutrient media.

The most significant differences in the complexes of micromycetes of lowland and mountain forests are revealed at the level of dominant species. Thus, in the group of species predominated in the complexes of soil micromycetes of at least one of the studied habitats in the lowland forests, there are: *Alternaria alternata* s. lat., *Aspergillus aculeatus*, *A. flavipes*, *A. japonicus*, *A. melleus*, *A. phoenicis*, *A. raperi*, *A. sydowii*, *Aureobasidium pullulans* var. *melanogenum*, *Cladosporium cladosporioides*, *C. oxysporum*, *Clonostachys byssicola*, *Eupenicillium shearii,*

Fusarium merismoides, F. solani, Gliocladium penicillioides, Gongronella butleri, Gonytrichum macrocladum, Heterocephalum aurantiacum, Idriella lunata, Microdochium bolleyi, Microsphaeropsis paliformis, Penicillium atrofulvum, P. brevicompactum, P. canescens, P. citrinum, P. daleae, P. decumbens, P. dierckxii, P. herquei, P. implicatum, P. miczynskii, P. ochrochloron, P. sclerotiorum, P. tropicoides, Pestalotiopsis spp.*, Phoma leveillei, Pochonia chlamydosporia, Pseudallescheria boydii, Purpureocillium lilacinum, Talaromyces aculeatus, T. diversus, T. flavus, T. ruber, T. rugulosus, T. verruculosus, Trichoderma harzianum, Trichosporon sporotrichoides*. There are either cosmopolitan species or species common mainly for regions with a hot climate. *Purpureocillium lilacinum* and *Trichoderma harzianum* are the species of particular note. The first species dominated only in the lowland forests; in the mountains, it was much less common. The second species was common everywhere.

The group of species, dominant in complexes of soil micromicetes at least one of the studied habitats in mountain forests, consists of: *Aspergillus phoenicis, Chaetomium fusiforme, Paecilomyces clavisporus, Penicillium aurantiacobrunneum, P. citreonigrum, P. citrinum, P. glabrum, P. implicatum, P. johnkrugii, P. lapidosum, P. raciborskii, P. sclerotiorum, P. spinulosum, Talaromyces funiculosus, T. loliensis, T. purpurogenus, P. waksmanii, P. wellingtonense, P. westlingii, Pestalotiopsis* spp.*, Phoma leveillei, Trichoderma ghanense, T. hamatum, T. harzianum, Umbelopsis angularis, U. isabellina*, light-colored and dark-colored sterile forms. Two species *Penicillium waksmanii* and *Trichoderma harzianum* dominated in three habitats, the rest - only in 1÷2 places. The predominance of *Penicillium* species in this group is noticeable, the genus *Aspergillus* is represented by only one species. Fungi from the order Hypocreales are absent among the dominant species.

The main functional role of soil micromycetes is participation in the decomposition of plant residues. According to the results obtained during the work, it can be propose that in mountain forests, where the humidity is higher and the seasonality is less pronounced, the contribution of fungi to the degradation of leaf litter is higher than in the lowland forests.

In monsoon forests with a pronounce seasonality, leaf litter occurs mainly during the dry period, when the activity of fungi is limited by the low moisture of the substrate. Thus, soil saprotrophs, representatives of the genera *Penicillium* and *Aspergillus* are most typical for soil, and another group of species predominantly associated with plants (phytopathogenic and epiphytic) is formed on the leaf litter. They begin the processes of decomposition, then the soil species settle the leaf litter, the speed of this depends on the availability of water.

By the degree of similarity between the complexes of micromycetes of the soil and the leaf litter of the studied habitats, authors can discuss about the degree of the stages of decomposition process. So, a study conducted in the spring of 2011 showed that the fungi of the soil and litter in the lowland forest differ quite clearly, the decomposition processes are at the initial stages and the soil fungi have not yet reach the litter. In the polydominant mountain rain forest (1100 m above sea level), the difference between the complexes of micromycetes of soil and litter is not so noticeable, and the soil species began to colonize the fallen leaves. In the mountain range (1990 m above sea level), the complexes of the micromycetes types of soil and litter are similar. In a place where the temperatures were lower and the humidity was higher, litter decomposition proceeded somewhat differently than in the lower forests, and at the time of work soil fungi had already begun to actively colonize litter on the soil surface.

In the conditions of lowland forests in the dry season, the growth of fungi on the leaf litter is inhibited by a lack of moisture, and at the beginning of the wet season the amount of litter is significantly reduced due to the activity of insects, in the first termites, which rapidly carry out and process the main share of leaf litter [3]. It can be suppose that the contribution of microscopic fungi to the destruction of plant residues in the lowland forests is somewhat less than in mountain forests, where the availability of water is higher for the entire season and the number of insects utilizing litter is lower.

Thus, the dependence of the structure of the complexes and the functional role of the soil-bearing microscopic fungi of the tropical forests of Vietnam on microclimatic factors due to the altitude of the habitat above sea level is shown in the work.

4. CONCLUSION

The main functional role of soil micromycetes is participation in the processes of destruction of plant residues. On the litter in the process of its decay, the composition of micromycetes changes from epiphilic and phytopathogenic to typical soil saprotrophs. Authors studied the complexes of cultivated micromycetes in soil, leaf litter and "air soil" in forests of different types. As a rule, the number and species diversity of micromycetes in soil is the lowest, and in "air soil" is the highest, Authors can propose that microbiological processes are most intensive there, and "air soil" is a kind of "hot spot" for the growth of micromycetes and other micro - and mesoorganisms.

The differences in the species composition of the micromycetes of the soil and litter in mountain forests are less than in the lowland ones because in mountain forests the hydration regime is more equal and the substrate moisture is (on average) higher. That is why the succession stages of fungi on the litter in mountain forests are faster then in lowland ones. According to the results obtained during the work, it can be propose that in mountain forests, where the humidity is more and the seasonality is less pronounced, the contribution of fungi to the destruction of litter is higher than in the lowland forests.

As a result of this work, a collection of pure cultures of micromycetes is collected. This collection includes 1614 strains of 430 species isolated from different substrates from the different types of forests. Potential of this cultures as a producers of biologically active substances valuable for pharmacology and biotechnology is a direction of author's further researches.

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TÓM TẮT

ĐA DẠNG VI NẤM TRONG HỆ SINH THÁI RỪNG VIỆT NAM VÀ VAI TRÒ CỦA CHÚNG TRONG PHÂN HỦY THẢM RỤNG THỰC VẬT

Rừng nhiệt đới đặc trưng bởi đa dạng các loài nấm. Trong nghiên cứu này tiến hành phân tích đa dạng vi nấm đất bằng phương pháp nuôi cấy Waxman trên môi trường agar. Kết quả nghiên cứu tại 13 Vườn Quốc gia và Khu Bảo tồn thiên nhiên của Việt Nam, đã phát hiện 492 loài thuộc 136 chi vi nấm phát triển trong đất và trên các giá thể liên quan tới đất.

Dựa trên những số liệu thu được, có thể tiến hành phân tích so sánh ban đầu tính đa dạng vi nấm đất các vùng khác nhau của Việt Nam.

Trong nghiên cứu này, lần đầu tiên phát hiện đặc trưng về cấu trúc thành phần loài vi nấm đất của rừng nhiệt đới khi so sánh với rừng ôn đới. Đã quan sát thấy: 1) tính đa dạng loài và đa dạng phân loại cao hơn; 2) đa dạng loài và số lượng các đại diện ngành Zygomycota thấp hơn; 3) phần lớn vi nấm thuộc bộ Hypocreales; 4) sự tham gia đáng kể của các loài nấm bộ Xylariales vào đa dạng loài nói chung của vi nấm đất; 5) tính đa dạng cao của chi Aspergillus và các bào tử của chúng; 6) lượng lớn các vi nấm có khả năng gây bệnh ở thực vật; 7) tính đa dạng và phong phú cao các dạng hình thái nấm không mang bào tử trên các môi trường tổng hợp.

Vai trò chức năng chủ yếu của vi nấm đất - là sự tham gia vào quá trình phân hủy mảnh vụn thực vật. Theo kết quả nghiên cứu, có thể giả thiết rằng, trong rừng vùng núi, nơi có độ ẩm cao hơn và tính mùa ít thể hiện hơn, vai trò của nấm trong việc phân hủy lá rụng cao hơn so với ở rừng đồng bằng. Trong các điều kiện ở rừng đồng bằng, vào mùa khô, sự phát triển của nấm trên lớp thảm rụng bị cản trở bởi thiếu ẩm còn vào đầu mùa mưa, lượng lá rụng giảm mạnh do hoạt động của côn trùng.

Từ khóa: Vi nấm đất, rừng nhiệt đới, da dạng sinh học, soil microscopic fungi, tropical forest, biological diversity.

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