

PhD THESIS

**Mitosporic fungi occurring on medicinal and aromatic
plants**

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INTRODUCTION, MAIN OBJECTIVES

In Hungary the cultivation of medicinal and aromatic is becoming widespread in horticulture. This is explained by the introduction of new and popular plant species and on the other hand by the expansive utilization of these species in nutrition and in health care. The restriction of the use of synthetic pesticides rises the demand of the use of plant protection products containing natural agents (eg plant extracts).

The increasing role of this horticultural sector and the spread of quality assurance standards in the production makes it essential to work out the principles of the protection of medicinal and aromatic plants. For this purpose as a first step the pathogens occurring on these plants must be revealed and disease cycles must be described. The role of diseases caused by fungi in medicinal and aromatic plant production is often underestimated. In spite of the fact that the role of these plants in nutrition, in health care and in synthetic pesticide free plant protection is increasing only a few researchers deal with their pathology. That seems a paradox. There is very little knowledge available about fungi infecting medicinal and aromatic plants, in many cases even their taxonomy is not clarified.

In Hungary the diseases of medicinal and aromatic plants are not known or fell into oblivion. Data about pathogens infecting medicinal and aromatic plants can be found mainly in the comprehensive works of the noted micologists László Hollós and Gusztáv Moesz. These works were printed in the first half of the 20th century, however, in most cases detailed descriptions are not available. The data are difficult to access, usually incomplete and neither are demonstrated by figures nor confirmed by microscopic measurements. Therefore they can not serve as a basis of the development of plant protection principals. In the 1970's and 1980's Ferenc Nagy and József Vörös dealt with the revealing of the phytopathological situation of some large scale grown plant species.

According to our observations among the different pathogens mitosporic fungi influence the most the production of medicinal and aromatic plants so in the dissertation the description of these fungi has been aimed.

The research was carried out in the field in various regions and climatic conditions in Hungary and in the laboratory of the Department of Plant Pathology in the Corvinus University of Budapest. In the survey the major cultivated and collected medicinal and aromatic plants were involved and mitosporic fungi occurring on them in field conditions were investigated.

The main objectives were as follows:

- To reveal mitosporic fungi infecting medicinal and aromatic plants;
- To clarify nomenclature and taxonomy of the pathogens;
- To describe symptoms;
- To assess disease incidence on cultivated medicinal and aromatic plants;
- To describe morphological and cultural characters;
- To reveal disease development.

MATERIALS AND METHODS

In recent years mitosporic fungi occurring on medicinal and aromatic plants have been investigated in the field in various regions and climatic conditions at Budapest-Soroksár and Herencsény in Hungary and in laboratory. Occasional studies were carried out in the Buda mountains, at Dunaföldvár, Fajsz and Szirák.

Symptoms were visually studied on the spot and later on collected plant samples in laboratory by stereomicroscope.

Disease incidence was assessed at Budapest-Soroksár and Herencsény. Plant parts were classified into disease categories which were modelled according to the types of symptoms. Disease indices were calculated. Disease incidence was assessed on the bases of disease indices and the frequency of symptoms.

Occurrence of fungi on seeds were assessed on wet filter paper and on agar media according to the relevant standards. In certain cases surface desinfestation was applied. The correlation between occurrence and seed germination was evaluated using a computer software. Influence to the emergence was assessed by germinating the seeds in soil in greenhouse and in plant growth chamber.

Overwintering of conidial structures of three pathogens was investigated in laboratory and in outdoor conditions.

Morphological characters were described by the microscopical measurements of conidial structures. For the description of cultural characters pathogens were isolated on agar media and pure or single spore cultures were obtained.

Cultural growth was investigated keeping the colonies in different temperatures. Micelial growth was measured regularly.

Taxonomic clarification of *Phoma*-like species was carried out on the basis of conidial septation *in vivo* and *in vitro* and that of the color reaction of cultures adding a drop of NaOH near the growth margins. Pathogenicity of the investigated fungi was tested on plants grown in pots and on living plant parts kept in Petri-dishes.

RESULTS

On the 33 investigated medicinal and aromatic plant species 32 mitosporic fungi have been identified belonging to 15 genera: 1 *Phyllosticta* sp., 1 *Microsphaeropsis* sp., 2 *Phoma* spp., 1 *Phomopsis* sp., 3 *Ascochyta* spp., 6 *Septoria* spp., 1 *Colletotrichum* sp., 1 *Botrytis* sp., 1 *Verticillium* sp., 7 *Ramularia* spp., 2 *Passalora* spp., 1 *Cercospora* sp., 1 *Dendryphion* sp., 2 *Alternaria* spp., 1 *Fusarium* sp. and its special form.

Suggestion has been made for the use of *Ramularia levistici* Oudem., *Passalora puncta* (Lacroix) S. Petzoldt and *Alternaria solani* (Ellis & G.Martin) Sorauer legitim names. Because of the significant differences in morphological and cultural characteristics of the *Dendryphion penicillatum* (Corda) Fr. isolates originating from different opium poppy plant parts, further taxonomic and nomenclatural study is suggested. Changing the taxonomic position of *Ascochyta daronici* to *Phoma* is reasonable according to the obtained results. Occurrence of *Microsphaeropsis glycyrrhizicola* on cultivated licorice, *Ascochyta cretensis* on fructus of sweet fennel are established. It is ascertained that among *Passalora* spp. *P. depressa* infects angelica and *P. puncta* infects sweet fennel.

Mitosporic fungi occurring on medicinal and aromatic plants cause leaf spots, shoot-, umbel- and blossom blight and wilting. Symptoms appear usually in the second part of the vegetation period. However, symptoms caused by *Phyllosticta cruenta*, *Septoria melissae*, *Verticillium dahliae*, *Ramularia menthicola*, *Ramularia rubella* and *Dendryphion penicillatum* appeared very early in the season.

Phomopsis diachenii, *Ascochyta cretensis*, *Botrytis cinerea*, *Passalora depressa*, *P. puncta* and *Dendryphion penicillatum* have been found on seeds or fructus. Occurrence of *Botrytis cinerea* on the seeds of medicinal and

aromatic plants was common, however, it does not inhibit germinating significantly. Micelia of *Dendryphon penicillatum* causing significant death of opium poppy seedlings was found on the surface of the seeds.

In recent years, severe disease incidence has developed on medicinal and aromatic plants due to the infection of *Microsphaeropsis glycyrrhizicola* on cultivated licorice, *Phomopsis diachenii* on caraway, *Septoria melissae* on lemon balm, *Septoria origanicola* on oregano, *Septoria virgaurea* on goldenrod, *Botrytis cinerea* on sweet basil, *Verticillium dahliae* on southernwood, *Ramularia levistici* on lovage, *Ramularia menthicola* on spearmint and green ment, *Ramularia rubella* on curly dock, *Passalora depressa* on angelica and *Passalora puncta* on sweet fennel. Disease incidence was influenced in some cases by environmental conditions of the locations (eg: *Septoria origanicola*), and more rarely by the differences between growing seasons (eg: *Septoria lavandulae*). Susceptibility of plants to the pathogens varied.

Morphological characters of the investigated mitosporic fungi are described in details. 14 species produced their conidia in pycnidial conidiomata, 1 species in acervulus and 17 species on conidiophors. Characters of fruiting structures are usually in accordance with existing data, however, in some cases significant differences can be found. Among the 32 investigated mitosporic fungi 29 species were isolated on artificial media and cultural characters were described. Cultural characterization was carried out by determining the colour, the shape, the likeness of the surface and the growth rate of the colonies. Micelia of *Phyllosticta cruenta*, *Microsphaeropsis glycyrrhizicola*, *Phoma exigua* var. *exigua*, *Phomopsis diachenii*, *Ascochyta* spp., *Colletotrichum dictamni*, *Botrytis cinerea*, *Verticillium dahliae*, *Alternaria* spp., *Fusarium oxysporum* and *F. oxysporum* f. sp. *basalici* grew well or moderately on agar plates. *Septoria* spp., *Ramularia* spp. and *Passalora depressa* are difficult to culture on agar media, growth rates are very low.

On the basis of pathogenicity tests, it has been proved that *Phoma exigua* var. *exigua*, *Ascochyta rubiae*, *Septoria melissae*, *S. origanicola*, *Ramularia menthicola*, *Passalora depressa* and *Alternaria solani* can infect intact leaves. Micelia of *Phomopsis diachenii* and *Septoria grindeliicola* can penetrate into the plant tissue only through wounds. In case of *Botrytis cinerea*, wounding contributed to a more intensive disease development. *Verticillium dahliae*, *Fusarium oxysporum* and *F. oxysporum* f. sp. *baialici* soilborn pathogens infect plants through wounded roots. A rapid disease development was observed on hollyhock inoculated by *Phoma exigua* var. *exigua*, on madder

inoculated by *Ascochyta rubiae*, on sweet basil inoculated by *Botrytis cinerea*, on jimsonweed inoculated by *Alternaria solani* and on purple coneflower inoculated by *Fusarium oxysporum*.

New scientific results

Since the description of mitosporic fungi occurring on medicinal and aromatic plants is a little investigated field of the science, in the dissertation numerous new data have been described from which the followings are emphasized:

1. The description of *Phomopsis diachenii*, *Ascochyta cretensis*, *A. rubiae*, *Septoria grindeliicola*, *S. lavandulae*, *S. origanica*, *Cercospora guatemalensis* and *Fusarium oxysporum* f. sp. *basalici* are new records in Hungary.
2. From the already known pathogens in Hungary the following mitosporic fungi have been found on new hosts: *Phoma exigua* var. *exigua* (on hollyhock), *Botrytis cinerea* (on sweet basil), *Verticillium dahliae* (on sea wormwood and on southernwood), *Ramularia menthicola* (on green ment, on peppermint and on spearmint), *Passalora depressa* (on angelica), *Alternaria solani* (on downy thorn-apple) and *Fusarium oxysporum* (on purple coneflower).
3. Occurrence of *Ascochyta rubiae* on madder, *Septoria grindeliicola* on Great Valley gumweed, *Verticillium dahliae* on sea wormwood and on southernwood, *Ramularia menthicola* on green ment and on spearmint and *Fusarium oxysporum* on purple coneflower have not been known in the literature so these are new records for the Science.
4. In case of some species - *Microsphaeropsis glycyrrhizicola*, *Ascochyta cretensis*, *A. doronici*, *A. rubiae*, *Passalora depressa*, *P. puncta* és *Dendryphion penicillatum* – suggestion has been made for their taxonomic position. The use of legitim names of the investigated mitosporic fungi has been clarified.
5. New types of symptoms were observed in case of *Microsphaeropsis glycyrrhizicola* (stem spots), *Ascochyta rubiae* (stem spots), *Septoria origanica* (sepal spots) and *Phomopsis diachenii* (umbel wilting).
6. The disease incidences caused by mitosporic fungi were assessed on cultivated medicinal and aromatic plants.
7. Significant difference was observed in susceptibility between hollyhock and common marshmallow to *Phoma exigua* var. *exigua* and between peppermint, spearmint and green ment to *Ramularia menthicola*.

8. It was found that *Septoria melissae*, *S. virgaurea* and *Ramularia menthicola* can overwinter as conidia.
9. Among the investigated 32 mitosporic fungi the description of cultural characters of 19 pathogens are new records for the Science.
10. In case of *Septoria lavandulae*, *S. melissae*, *S. virgaurea* and *Fusarium oxysporum* f. sp. *basalici* the optimum temperature for micelial growth was determined.
11. On the bases of the results of the pathogenicity tests in more pathogen-host relationships the opportunity of direct infection has been proved. In case of some pathogens their opportunistic wound parasitic character has been demonstrated.

DISCUSSION AND CONCLUSIONS

Incidence of mitosporic fungi on medicinal and aromatic plants can be observed frequently. They often cause significant damage. Susceptibility of host plants to the pathogens varies. Disease incidence was influenced by the environmental condition of the location and the differences between growing seasons only to a low extent. At the locations the lack of the knowledge of the pathogens and so the lack of plant protection techniques often play some role in the development of the disease incidence.

So far, about mitosporic fungi occurring on medicinal and aromatic plants a comprehensive study has not been published in Hungary. Since the plant protection techniques in these plants are not well developed, an adaptable and precise characterization of fungal pathogens is essential. The results contribute to the development of a sustainable pest management against pathogens in medicinal and aromatic plants, and can serve as a basis for the further taxonomic clarification of mitosporic fungi occurring on these plants.

Since the dissertation is mainly descriptive certain topics could not be worked out in details. Completing taxonomic results with further investigations according to modern criteria is suggested.

As a first step the role of mitosporic fungi has been revealed but later on investigation should be extended to other types of pathogens.

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