

CURRENT STUDY ON THE PARAZITIC MICROORGANISMS OF THE MEDICINAL AND AROMATIC PLANTS IN SERBIA

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Keywords: medicinal and aromatic plants, diseases, fungi, fastidious bacteria, phytoplasma

INTRODUCTION

Medicinal plants are among the most economically significant plants in Serbia. There are over 700 varieties of these plants on the territory of Serbia contributing 19,65% of the total flora. (Sarić M., 1989). Of the 420 registered varieties, 279 are used commercially as medicinal and aromatic plants. In order to preserve medical plants in nature from over exploitation, they are grown as commercial crops in plantation. There is a long tradition of growing medical and aromatic herbs commercially such as mint, marsh mallow, sage, St. John's wort etc. in Serbia.

The commercial cultivation led to the occurrence and spread of the plant diseases and on some hosts (St. John's wort, coneflower), the diseases were of the epidemic proportions. The parasites of the medicinal and aromatic herbs were studied over 17 years and the summary of the findings is reported in this paper.

MATERIAL AND METHODS

The diseased plants were collected from the collection field at Institute for medicinal plant research in Pančevo, as well as from other areas in Serbia in the period of 1990 to 2005. The seeds, seedlings and adult plants are analysed for the presence of plant pathogens. The standard methods such as: seed germination on the filter paper (Mathur and Kongsdal, 2003), and isolation on the artificial medium were used. The identification of the pathogens was done according to their morphological characteristics. The pathogenicity of the most common isolated fungi was tested by Molt and Simone procedure (1967). Artificial inoculations of the test plants were done by using fragments of the mycelia or conidia suspension. Inoculated plants were kept in the wet chamber until the symptoms appeared. In some cases pathogenicity was tested by grafting fragments of the diseased plants on the healthy ones.

The differentiated centrifugation method was used to extract the pathogen from the root, stem and leaf of the St John's wort plants with the symptoms

of redness. The sections (50 and 100 nm) of the xylem tissue of the infected plants were made by using the ultramicrotome.

Preparation of the plant material was made by using Hopkins et al. procedure (1973). The sections were observed by the electron microscope. For the identification of the causers of redness of *Echinacea* sp. PCR method was used. The fragments of the diseased plants were grafted on the healthy ones.

RESULTS AND DISCUSSION

The diseases of the mint plant (*Mentha piperita*)

The numerous pathogens of the mint plant have been described, and fungi are the predominant. The leaves of the cultivated mint were severely affected by the rust (*Puccinia menthae* Pers.) on almost all location in Serbia. The leaves of such diseased plants are useless for the pharmaceutical purposes.

Among the eight species of the stolone-born fungi the most important are the ones belonging to the genus *Fusarium* and *Verticillium* which causes specific chloroses and necroses on the infected plant tissues. The following species were determined: *Fusarium equiseti*, *Fusarium semitectum*, *Fusarium subglutinans*, *Verticillium lateritum*, *Verticillium dahliae*, *Penicillium* spp., *Trichotecium roseum* and *Alternaria* spp. The presence of these pathogens was 1-8% only (Pavlović et al., 2000).

According to the Russian data (Dobrzakova et al., 1956) the main pathogens of mint are *Fusarium* spp., *Septoria menthae*, *Septoria menthicola*, *Ramularia menthicola*, *Erysiphe cichoracearum* f. *menthe* and *Peronospora sigmaticola*. The leaves attacked by those fungi could not be sold or used for further production.

The diseases of the valerian plant (*Valeriana officinalis*)

Twelve species of fungi were identified on the samples of the diseases valerian: *Alternaria alternata*, *Phoma* spp., *Peronospora valerianae*, *Fusarium oxysporum*, *Fusarium sporotrichoides*, *Fusarium solani*, *Sclerotinia sclerotiorum*, *Epicoccum purpureascens*, *Verticillium* spp., *Penicillium* spp., *Mucor* spp. and *Aspergillus* spp. (Pavlović, 2003). Among the leaf and root pathogens the following fungi were prominent: *Fusarium*, *Alternaria* and *Phoma*.

The diseases of the St. John's wort (*Hypericum perforatum* and *H. barbatum*)

On the St. John's wort seeds ten species of fungi were identified: *Alternaria* spp., *Aspergillus* spp., *Fusarium oxysporum*, *Fusarium solani*, *Fusarium semitectum*, *Fusarium proliferatum*, *Penicillium* spp., *Epicoccum purpurascens*, *Verticillium* spp., *Mucor* spp. (Pavlović et al., 2000a). Fungi that belong to genus *Alternaria*, *Penicillium*, *Acremoniella*, *Aspergillus* and *Epicoccum* were always present in the samples, but in less intensity. Four species of the genus *Fusarium* were often present on the St. John's wort seeds, causing the death of the seeds or wilting of the seedlings.

Specific symptoms were observed on the St John's wort a few years ago (Pavlović et al., 2004). The leaves and stems of diseased plants became reddish the tissue necroted and the infected plants wilted and died before the vegetation, sometimes before flowering. On the some fields there were more than 90% of the diseased plants.

The analysis of the ultrathin vascular tissue sections of the diseased plants with the redness symptoms, the rod-shaped organisms were observed. They belonged to the group of the fastidious bacteria of the rickettsia type, based on their morphology and Gram staining.

Grafting fragments of the diseased plants onto the healthy ones caused successful reduction on the symptoms. A large number of leafhoppers as the potential vectors were collected and their identification is currently under way.

From the plants with the redness symptoms *Fusarium* and *Colletotrichum* were isolated (Ivanović et al., 2002). However, artificial inoculation of the St John's wort plants under the laboratory conditions with *Fusarium* and *Colletotrichum* isolates, did not produce the characteristic symptoms of the redness and drying / wilting.

The literature search shows no data on the fastidious bacteria on the aromatic herbs, therefore our study is the first one to establish the presence of this pathogen in the vascular tissue of the St John's plants (Pavlović et al., 2004, 2005). However, the phytoplasma have been described as a pathogen causing same symptoms (Bruni et al., 2005).

It is very important to study the redness disease on the aromatic plants as they spread rapidly and affect a number of hosts, causing a large reduction in yield. These symptoms have also been identified on the oregano (*Origano vulgare*), lovage (*Levisticum officinale*) and dandelion (*Taraxacum officinale*), (Pavlović et al. 2004a).

The diseases of the coneflower (*Echinacea purpurea* and *E. angustifolia*)

Echinacea species started to be grown commercially as plantations in Serbia in 1998. The investigation on the health status of the seeds of

E. purpurea and *E. angustifolia* has shown the presence of the numerous parasites. The following fungi have been identified: *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium proliferatum*, *Epicoccum purpurascens*, *Phytium* spp., *Rhizoctonia solani*, *Penicillium* spp., *Aspergillus* spp., and *Botrytis cinerea*.

The literature studied quotes these fungi *Sclerotinia sclerotiorum*, *Alternaria* spp., *Botrytis cinerea*, *Fusarium* spp. and *Phythium cichoracearum* (Sholberg et al., 1999).

Recently of type of phytoplasma has been identified as a serious and most destructive pathogen *Echinacea* spp. in the USA and Canada. (Hwang et al., 1997). The first symptoms of the disease on the cultivated *Echinacea* spp. in Pančevo and Indjija were notified after the second year of growing. The initial symptoms were yellowing and then redness of the lower leaves. The disease spread quickly, affecting 30% of the plants. The leaves of the diseased plants were smaller, wilted and dried. The redness spread on the stem, which showed smaller internodes and a reduction in growth, giving plants the appearance of the bush. Sometimes the plants died. The flowers showed proliferation and virescence. The electromicrographs obtained from the ultrathin sections of the vascular tissue of the diseased plants, showed the presence of the phytoplasma with oval to spherical shape, without the cell wall and surrounded by a single membrane (Pavlović et al., 2004). The PCR identification at University of Udine, Italy, recently confirmed that this pathogen of the *Echinacea* belongs to the Stolbur type of the phytoplasma.

The diseases of the yellow gentian (*Gentiana lutea* L.)

The cultivation of the yellow gentian in Serbia has started recently. The pathogens cause the changes on the seeds such as colour change, withering, spots and reduced germination. The following pathogens on the gentian seeds were identified: *Alternaria alternata* (78%), *Fusarium oxysporum* (5%), *Botrytis cinerea* (3%), *Aspergillus flavus* (3%), *Penicillium* spp. (3%) and *Epicoccum purpurascens* (2%).

The economic damage is caused by the reduction in the seed germination. When the seeds were affected by the *Alternaria* they did not germinate at all. The above mentioned pathogens are also common on the seeds of balm, sage, feverfew, valerian, St. John's, conewlover, camomila. (Kostić et al., 2004).

The diseases of the balm (*Melissa officinalis* L.)

The literature studied gives little information about the pathogens affecting the seeds. A long term study of the balm seeds quality identified the presence of the diseases in the early stages of the seed development, causing regular decay of seedlings, decreasing the percentage of germination and, ultimately, the decrease of the number of plants per area unit. The following fungi were isolated: *Alternaria tenuis*, *Aspergillus niger*, *Botrytis cinerea*, *Cephalosporium* spp., *Septoria* spp. *Epicoccum*

purpurascens, *Fusarium* spp., *Mucor* spp. and *Penicillium* spp. (Pavlović S., 2001).

The disease of the sage (*Salvia officinalis*)

The most common disease of the green parts of salvia is powdery mildew, and in the case of a severe attack, the diseased leaves become yellow and fall off. The leaves covered by the white-grey film lose their value as the source of aromatic oils. The following fungi were identified on the seeds and leaves of salvia: *Fusarium moniliforme* var. *subglutinans*, *Fusarium equiseti*, *Fusarium* spp., *Cercospora savicola*, *Alternaria* spp., *Botrytis cinerea*, *Chaetomium* spp., *Doratomyces* spp., *Epicoccum prupurascens*, *Penicillium* spp., and *Verticillium* spp. (Kostić et al. 1999).

Pironethe (1996) cite these pathogens as well as *Pythium debaryanum*, *Pellicularia filamentosa*, *Cercospora salvicola*, *Ramularia salvicola*, *Puccinia caulicola*, *Puccinia farinacea*, *Puccinia salvicola* and *Peronospora lamii*.

Diseases of marshmallow and mallow (*Althea officinalis* and *Malva silvestris*)

Puccinia malvacearum is a significant pathogen of the leaves of marshmallow and mallow in Serbia. The severely infected leaves were completely destroyed (Pavlović et al., 2002). The following fungi were isolated from the ungerminated seeds or the withered germ tubes of the marshmallow plants: *Fusarium* sp., *Alternaria* sp., *Epicoccum* sp., *Penicillium* sp., and *Aspergillus* sp., were isolated. Three *Fusarium* species (*F. oxysporum*, *F. semitectum* and *F. sporotrichoides*) (Pavlović and Stojanović, 2002) and *Sclerotinia sclerotiorum* (Pavlović and Stojanović, 2001) were detected in the necroted tissue of the root and stalk of the marshmallow.

The diseases of camomile (*Chamomilla recutita*)

Camomile is a very important cultivated aromatic plants in Serbia. Luckily, this plant is not significantly affected by pathogens. The following fungi are detected on the seeds: *Alternaria* sp., *Penicillium* sp., *Aspergillus* sp., *Verticillium* sp., and *Fusarium moniliforme* (Pavlović and Dražić, 2000). Only *F. moniliforme* can cause the damage during the germination under the certain conditions.

CONCLUSION

Medicinal and aromatic herbs proved to be suitable hosts for a large number of pathogens, mainly fungi from the following genus: *Fusarium*, *Alternaria*, *Penicillium*, *Aspergillus*, *Verticillium*, *Sclerotinia*, *Puccinia*, *Phoma*, *Colletotrichum*, *Septoria* etc. The majority of these fungi affect the seed germination and their vitality. They often cause spots, wilting and rust on the green parts of the plant.

Sclerotinia sclerotiorum is a significant pathogen on more than 400 plants, including those

mentioned in this study. Considering that this fungus can survive over five years in the soil, it is important to observe the rules of rotation during the cultivation of the aromatic herbs.

The herbs mostly affected by the disease are St. John's and coneflower, where up to 90% of the plants show the symptoms. As all affected plants die before the end of vegetative season, the presence of the fastidious bacteria in St John's wort and the phytoplasma in coneflower, can significantly the cost-effectiveness of their commercial growth.

The following aspects will be subject to the future study: the identification of the molecular structure of the fastidious bacteria and phytoplasma, as well as the identification of the vectors and the host affected.

ABSTRACT

Pathogenic microorganisms on medicinal and aromatic plant species cultivated on plantation in Serbia were studied during the last ten years. The seeds of majority of these plants were contaminated with fungi from the genus *Fusarium*, *Alternaria*, *Verticillium*, *Colletotrichum*, *Penicillium*, *Aspergillus*, *Rhizopus* and *Phoma*, which significantly reduced seed germination and caused seedlings decay. On the stem and leaves the symptoms of powdery mildew rust, fleckening and wilting were manifested. The fungi from the genus *Oidium*, *Puccinia*, *Coleosporium*, *Phragmidium*, *Alternaria*, *Fusarium*, *Phoma*, *Septoria*, *Sclerotinia*, *Verticillium* and *Botrytis* were isolated as the causative agents. The most intensive changes were recorded on St. John's wort and coneflower, where percentage of the diseased plants was very high. In the tissue of the diseased *Echinacea* species with the symptoms of yellowing (witches brooms, virescence), the presence of the phytoplasma (Stolbur type) was established. In the xylem vessels of the St. John's wort diseased plants, with the symptoms of redness and remature decay, the presence of fastidious bacteria (rickettsia – like organisms – RLO) was recorded. The symptoms of redness were also registered in the cultivated oregano and lovage, as well as in the wild-growing dandelion.

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