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ANTIFUNGAL ACTIVITY OF ESSENITAL OIL *HYSSOPUS OFFICINALIS* L. AGAINST MYCOPATHOGEN *MYCOGONE PERNICIOSA* (MANG)*

ABSTRACT: The most commonly cultivated mushroom species is the *Agaricus bisporus* Lange (Imb). One of the major pathogenic diseases of the cultivated mushroom in Serbia is *Mycogone perniciosa* (Mang). Biological control systems are not much used in mushroom cultivation. Medical and aromatic plants have been placed in the focus of intense studies.

Pure culture of the *M. perniciosa* was isolated from infected *A. bisporus*. The essential oil of *Hyssopus officinalis* L. is used as a potential antifungal agent. The most abundant components in oil are isopinocamphone (43.29%), pinocamphone (16.79%) and b-pinene (16.31%). Antifungal activity of Hyssop was investigated by the modified microatmosphere method. The minimal inhibitory quantity was 5 μL/mL and a minimal fungicidal quantity was 15—20 μL/mL.

There is no report on the use of Hyssop essential oil in mushroom disease.

KEY WORDS: *Agaricus bisporus*, *Hyssopus officinalis*, essential oil composition, antifungal activity, *Mycogone perniciosa*

INTRODUCTION

Agaricus bisporus Lange (Imbach) is a common edible mushroom with major economic value and a cosmopolitan distribution (Kerrigan, 1995). World wide cultivation of the button mushroom is 1.9 million tones in 1998/

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1999 (Van Griensven, 2003). The cultivated mushrooms are subject to various diseases and pests that have the capacity to cause serious crop losses. Many microorganisms, such as fungi, bacteria and viruses attack mushrooms. Fungi are effectively the most important group of pathogens (Fletcher et al., 1986). One of the major pathogenic diseases of the cultivated mushroom is *Mycogone perniciosa* (Mang), commonly known as Wet Bubble Disease (WBD) which caused considerable crop loss (Sisto et al., 1997, Sharma and Kumar, 2000, Bora and Özaktan, 2000, Nanagulyan and Yesayan, 2002).

The symptom of WBD is the development of cauliflower-like distortion on fruit bodies of *A. bisporus* like sclerodermoid masses that are white and fluffy at the beginning, but become brown with age and decay. In the conditions of a very high humidity brown drops develop on the surface of tumour-like bodies. Spores are infectious and spread by water splash and by insect vectors. The primary source of the pathogen is contaminated casing, but the secondary one is caused by facilities, personnel and insect vectors.

Mushroom cultivation in Serbia is still less developed than in other European countries. *M. perniciosa* has a significant influence on the quality and yield of mushrooms.

Very limited numbers of fungicides are available and approved for use in mushroom cultivation. Also, the development of pathogen resistance to the fungicides was closely related to the frequency of their use (Grogan and Gaze, 1998). Biological control systems, which have been successfully applied to some crops, are not much used in mushroom cultivation. It is difficult to find some safe disease spray to use on mushrooms when they are close to harvest. One possibility might be using herbal spray. Medical and aromatic plants have been placed in the focus of intense studies.

Hyssopus officinalis, L. belongs to the Labiate family of plants, of which numerous species have antiseptic properties against bacteria, fungi and viruses. Hyssop is a perennial sub shrub native to southern Europe, the Mediterranean region, and temperate Asia and naturalized in the United States. The therapeutic activity of the herb of *H. officinalis* has usually been attributed to the components of its essential oil. As a medicinal plant, Hyssop has been used as a carminative, diaphoretic, emmenagogue, expectorant, stimulant, stomacher, and tonic. Leaves have been used as a remedy for asthma, rheumatism, sore throats, wounds, ulcers, and tumours (Lawless, 2002).

In this study, we examined the action of essential oil of *Hyssopus officinalis* against the mycopathogen *Mycogone perniciosa*. The use of natural antifungal compounds in the control of human, animal and plant diseases of microbial origin was reported before (Soković, 2001).

MATERIALS AND METHODS

Essential oil and analysis — Essential oil of *Hyssopus officinalis* L. is commercial sample obtained by the Institute for Medicinal Plant Research „dr Josif Pančić", Belgrade.

Essential oil was investigated for its composition by the use of analytical GC/FID and GC/MS technique. For these purpose HP 5890 series II gas chromatograph, equipped with split-split less injector, fused silica capillary column (25 m x 0.32 mm), coated with cross-linked methyl silicone gum (0.5 μm film thickness), and FID was employed. Essential oil solutions in ethanol (1%) were injected in split mode (1:30). Injector was heated at 250°C, FID at 300°C, while column temperature was linearly programmed from 40—280°C (4°/min). GC/MS analyses were carried out on a HP-GCD, equipped with split-split less injector, fused silica capillary column (50 m x 0.2 mm) PONA, coated with cross-linked methyl silicone gum (0.5 μm film thickness). The chromatographic conditions were as above. Transfer line (MSD) was heated at 280°C. EIMS spectra (70eV) were acquired in scan mode in m/e range 40—300.

The identification of individual constituents was made by the comparison of their retention times with those of analytical standards, and by computer searching, matching mass spectral data with those held in Wiley/NBS library of mass spectra. For quantification purposes area percent reports obtained by FID were used (Adams, 1995).

Fungal strain and media — Samples of diseased mushrooms were collected from mushroom farms in Serbia. Pure culture of the *M. perniciosa* was isolated from diseased *A. bisporus* in the Mycological Laboratory, Institute for Biological Research „Siniša Stanković". The mycopathogen was maintained on potato dextrose agar (PDA). The cultures were stored at 4°C and subcultured once a month (Booth, 1971).

Test for antifungal activity — The modified microatmosphere method (Zollo et al. 1998) was used for the investigation of antifungal activity of essential oils. Petri plates measuring 50 mm were filled with 10 mL potato dextrose agar (PDA) medium and then seeded with a small amount of 7-days--old mycelium culture of the tested fungi. The Petri dishes were then inverted and the determined amount (5—20 μL/mL) of pure oils impregnated on sterile filter paper discs (6 mm in diameter) deposited on the inverted lid. Minimal inhibitory quantities (MIQ) and minimal fungicidal quantities (MFQ) of essential oils were noted every 7 days. MIQ and MFQ are reported as the mean ± SD of three replicates for each concentration (quantities) of oils. The inverted Petri dishes were incubated at 25°C for 21 days.

RESULTS AND DISCUSSION

The results of chemical analysis of essential oil from Hyssop are presented in Table 1. The results suggested that the activity of *H. officinalis* can be attributed to ketons which are the main constituents: isopinocamphone (43.29%), pinocamphone (16.79%) and b-pinene (16.31%).

The essential oil of *H. officinalis* showed a very strong antifungal activity. The minimal inhibitory quantity was 5 μL/mL and minimal fungicidal quantity was 15—20 μL/mL.

Furthermore, antimicrobial activities of *Hyssopus* species were shown in other previous studies (Mazzanti et al., 1998, Renzini et al., 1999). The essential oil of Hyssop had fungistatic action on *Aspergillus fumigatus*. Binding of this oil or some of its constituents to membranes has been found to affect ion exchanges (Ghfir et al., 1994.) The antifungal and fungicidal effects of Hyssop oil and its individual components were studied against plant pathogenic fungi (Letessier et al., 2001). The very strong antifungal potential of Hyssop essential oil can be explained by high amount of ketons which are the main constituents (Griffin et al., 2000).

In the control of WBD Prochloraz manganese, Benzilmidazole fungicides and 1% formalin in treating casing give a good control, but *Mycogone* remains a constant threat. The high toxicity of these fungicides and emerging tolerance of mycoparasites to some fungicides makes it necessary to continue the search for new antifungal substances. During the past years numerous antifungal agents have been formulated and evaluated for use in the management of fungal diseases microwave treatments or some effective antagonistic bacteria (Bora and Özaktan, 2000). Essential oils used in this work could be a very good alternative in treatment of fungal diseases because of their very good antifungal activities.

Table 1. The composition of essential oil of *Hyssopus officinalis.*

Components	*H. officinalis* %	RI
α-thujene	0.25	307
α-pinene	0.95	319
camphene	0.24	340
β-pinene	16.31	386
myrcene	0.54	408
p-cymene	1.07	471
limonene	1.56	481
1,8-cineole	0.49	485
trans-ocimene	0.54	519
γ-terpinene	0.93	545
terpinolene	0.26	608
α-thujone	0.13	642
β-thujone	0.13	667
pinocamphone	16.79	775
isopinocamphone	43.29	809
myrtenal	1.37	864
methyl chavicol	0.07	869
β-bourbonene	1.20	1355
β-elemene	0.09	1375
α-gurjunene	0.18	1421
β-caryophyllene	1.00	1442
allo-aromadendrene	0.63	1546
germacrene D	0.93	1594
bicyclogermacrene	0.12	1632
RI-DB 5		

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АНТИФУНГАЛНА АКТИВНОСТ ЕТАРСКОГ УЉА *HYSSOPUS OFFICINALIS* L. НА МИКОПАТОГЕН *MYCOGONE PERNICIOSA* (MANG)

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Резиме

Agaricus bisporus Lange (Imb) је најчешће комерцијално гајена јестива гљива. Различити микроорганизми гљиве, бактерије и вируси су изазивачи болести у гајилиштима шампињона. *Mycogone perniciosa* (Mang) је изазивач болести познате под називом влажни мехур и најчешћи узрочник губитака у гајилиштима у Србији. Биолошка контрола, која је успешно примењивана на неким пољопривредним културама, није коришћена приликом узгоја гљива. Једна од могућности је примена биљних спрејова. Лековите и ароматичне врсте биљака се интензивно истражују као могући антифунгални агенси.

Узорци оболелих шампињона су сакупљани у гајилиштима у Србији. Културе *M. perniciosa* су изоловане са оболелих плодоносних тела *A. bisporus*. Коришћено је етарско уље *Hyssopus officinalis*. Најзаступљеније компоненте уља су изопинокамфон (43.29%), транс-пинокамфон (16.79%) и б-пинен (16.31%). Антифунгална активност етарског уља изопа испитивана је модификованом „микроатмосфера”-методом. Минимална инхибиторна количина је била 5 μL/mL, а минимална фунгицидна количина 15—20 μL/mL.

Велик број препарата је направљен и примењен за контролисање обољења печурака: фунгициди, примена микроталаса или дејство неких антагонистичких бактерија. Досад није било саопштења о примени етарског уља изопа против изазивача болести гајених гљива.