EFFECT OF ESSENTIAL OILS ON MYCOPATHOGENS OF *AGARICUS BISPORUS*

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ABSTRACT

The control of mycopathogens in mushroom cultivation is based on the usage of very limited active substances, cultural protection practices and sanitation precautions. The lack of registered chemicals is a problem in the practice. Integrated disease management have been used in mushroom cultivation but there is a need for novel selective fungicides and cheap reliable disinfectants.The main target of the present work is to find natural products which can be tested in cultivation against the major *Agaricus bisporus* fungal diseases with effect of prevention and/or curative methods. In the trial essential oils of aromatic plants; *Bacillus subtilis* and active substance prochloraz-Mn were tested *in vitro.* The examined pathogens were *Clabobotryum dendroides, Mycogone perniciosa, Lecanicillium fungicola* and *Trichoderma aggressivum* f. *aggressivum.* The effect of essential oils on the growth of a cultivated hybrid variety of *A. bisporus* was also tested: (1) hole-test on agar plates; (2) contact test on agar plates; (3) volatile fraction test on agar plates and (4) test on mushroom fruit bodies. Among the tested oils *Cinnamonium, Mentha* and *Pelargonium* proved to be the most effective which completely inhibited the growth of all four mycopathogens. It can be concluded that *Citrus* and *Tyhmus* were not effective in this case. *Bacillus subtilis* were effective against all tested pathogens in contact test. Further research is necessary to determine correct application and concentrations, because almost all tested oils inhibited the growth of the cultivated mushroom hyphae as well. From that point of view, only *B. subtilis* looks prospective in cultivation.

Keywords: disease, integrated, *Mycogone*, *Lecanicillium*, mushroom

INTRODUCTION

A number of pests and diseases cause problems in white button mushroom (*Agaricus bisporus*) production, as they significantly reduce both the quality and quantity of mushrooms. Due to the short growing period, the special cultivation technology and the lack of available synthetic substances, the scope of applying any chemical against pests and pathogens is very limited [1].

There is a growing tendency of using biological plant protection products in vegetable production (e.g. tomato, pepper and cucumber), where high efficacy can be ensured with advanced climate control. This should serve as an example for mushroom production as well. Resistance against registered products can be observed in more and more cases [2, 3], thus widening the range of active substances is an urgent task. The diseases regularly present and causing loss of revenue in button mushroom cultivation are the following [1]: cobweb disease (*Clabobotryum dendroides*), wet bubble disease (*Mycogone perniciosa*), dry bubble disease (*Lecanicillium fungicola*) and compost green mould (*Trichoderma aggressivum* f. *aggressivum*).

In each case the first step of mushroom protection is prevention. Some producers apply chemicals regularly, instead of using them only when pests and pathogens are really present in the culture. To provide proper hygienic conditions from the beginning and to treat already infected mushroom cultures, we are seeking active agents that are selective, easy to license, inexpensive and easy to use in mushroom production. One of the possible solutions is to try essential oils, about which some data are already available [4-6]. The effect of essential oils of herbs on mushroom pathogens has been examined in Serbia. Their results showed that thymol, carvacrol and cimol, which are the main essential oil components of thyme, significantly inhibit the growth of *Lecanicillium fungicola* and *Trichoderma harzianum* [7]. The oils of peppermint

(*Mentha piperita*) and spearmint (*Mentha spicta*) could also block the growth of *Trichoderma harzianum*, *Verticillium fungicola* and *Pseudomonas tolaasii* [7]. The same effect can be observed in case of wet bubble disease [8-10]. In another study [11] the toxicity of lavender, anise, parsley, chamomile, fennel, geranium, oregano and common sage was tested against bubble diseases and cobweb disease. The main component of pelargonium (geraniol and citronellol) and oregano (carvacrol and thymol) had the strongest effect on pathogens [11]. Initial studies on the post-harvest application of essential oils on fruitbodies are being conducted [12]. However, no recommendations on components, technologies or dosage exist or available yet. It is especially important to come up with solutions since neither the selectivity of these oils, nor their possible antifungal effect on the mycelia of the cultivated mushrooms has been tested so far.

The main target of the present work is to find natural products which can be tested in cultivation against the major *A. bisporus* fungal diseases, with effect of prevention and/or curative methods. In the trial essential oils from aromatic plants; *Bacillus subtilis* and active substance prochloraz-Mn were tested *in vitro* to compare the commonly used active substances with novel ingredients.

MATERIALS AND METHODS

1. Materials

In this study the pure essential oils of an essential oil producing company (*Aromax*) were used. To compare efficiency a licensed active substance (prochloraz-Mn complex) and a bacterial product were applied parallel to the essential oils (Table 1).

Table 1. Essential oils and other active substances used for *in vitro* tests

Complex essential oils of	Other active substances
Cinnamonium zeylandicum (cinnamon)	*Bacillus subtilis* QST 713 strain ('Virtuoso')
Citrus aurantium (bitter orange)	Prochloraz-Mn, 50% ('Sporgon 50WP')
Matricaria chamomilla (chamomile)	
Mentha spicata (spearmint)	
Pelargonium graveolens (geranium)	
Salvia officinalis (common sage)	
Thymus vulgaris (common thyme)	

The pure pathogen cultures of the culture collection of the Corvinus University of Budapest, Department of Vegetable and Mushroom Growing were used for the purpose of this study. The tested pathogens were the following: *Cladobotryum dendroides; Mycogone perniciosa; Lecanicillium fungicola; Trichoderma aggressivum* f. *aggressivum. Agaricus bisporus* 'A15' was used as control for testing selectivity.

2. Methods

Hole-test

PDA media (Biolab, 39 g/l) were poured into petri-dishes (90 mm diameter), than with a corkborer (5 mm) 3 holes were cutin media. One hole was filled with 150 µl essential oil (0.01% concentration) while rest two holes with 150 µl sterilized DW. Other petri-dishes were filled with 150 µl prochloraz-Mn (0.5 g/l) solution or 150 µl *Bacillus* suspension (2 g/l). Each treatment had 4 replicates and were placed on 25 °C. After 10 days incubation a picture was taken with the characteristics of the developed mycelia.

Contact test

Into the PDA media (Biolab, 39 g/l) after autoclaving (121 °C, 20 min) chemicals were mixed still in liquid phase:

- Control: 1 drop Tween-20
- Essential oils: cc. 150 µl/l + 1 drop Tween-20
- Prochloraz-Mn: 0.5 g/l
- *Bacillus*: 2 g/l

After solidification in petri-dishes, 5 days old pure mycelial culture (diameter 7.5 mm) was placed into the center.Every treatment had four replicates. The petri-dishes were incubated on 25 °C for 10 days than colony diameters were measured.

Volatile fraction test

PDA media (Biolab, 39 g/l) were poured into petri-dishes than inoculated with 10 days old pure mycelia cultureand turned down. Into the lid a sterilized paper was laid and sprayed with 500 µl essential oil in 0.01% concentration. As a negative control 500 µl of prochloraz-Mn (0.5g/l) solution was also tested, sterilized distilled water was used as positive control. 500 µl of *Bacillus* (2 g/l) was also tested. Every treatment had four replicate. The petri-dishes were incubated at 25 °C for 10 days than colony diameters were measured.

Test on mushroom fruit bodies

The effect of the tested materials was also studied on fruitbodies. 100 µl of essential oils in two concentrations (cc., 1000× dilution); *Bacillus* (2 g/l) and prochloraz-Mn (0.5 g/l) as well in two concentrations (cc., 1000× dilution) were pipetted onto mushrooms than incubated 24 hours on room temperature and lesions were noticed.

RESULTS AND DISCUSSION

Results of Hole-test

The developed mycelia did not show the typical concentric shape, but it was distorted opposite the essential oil (figure not shown). In this type of test no differences in efficacy were registered, since all the essential oils diffused in the media and inhibited mycelial growth. The same effect was seen not only by each pathogen, but in case of the button mushroom as well. This result could mean that in practice an even more intensive translocation of the oils can be expected, since the cultivating media is less compact.

Results of Contact test

Fig. 1 shows the diameters of the mycelia growth 10 days after inoculation on poison agar plates. It can clearly be seen that cinnamon, spearmint and geranium essential oils were to be most effective in mycelial growth inhibition. Due to these oils, each pathogen stopped developing. Unfortunately the tested essential oils are not selective enough, since they blocked the button mushroom mycelia as well. Amongst the tested oils, thyme was the only one with selectivity: it did not affect the mycelial growth of button mushroom significantly, and in the meantime blocked the growth of *Cladobotryum*, *Mycogone* and *Trichoderma*.The figure shows a slight but not significant ($p<0.05$) positive effect in case of *Lecanicillium*, *B. subtilis* also proved to be effective. Results prove the contact effect of essential oils. They all have a certain level of strong inhibition. Spearmint can be characterised with partial selectivity: it blocked mycelia growth in all except one (*Lecanicillium*) case.

Results of Volatile fraction test

This type of setting is most comparable to a possible large scale application. The essential oils evaporate from the paper stripe placed into the Petri-dish. The inhibiting effect is just like in case of contact application of oils mentioned above.

Cinnamon, spearmint and geranium oils proved to be most effective inhibitors in case of each pathogen. Chamomile completely blocked the mycelial growth of the button mushroom too (Fig 2).

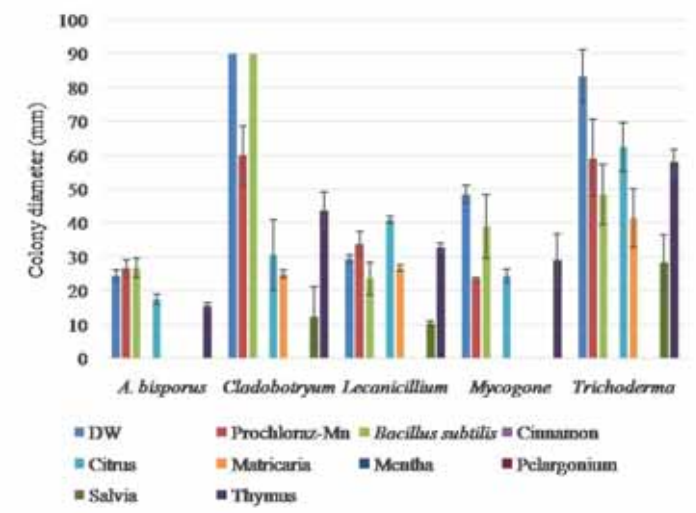

Figure 1. Influence of essential oils with poison agar technique on mycelial colony diameter

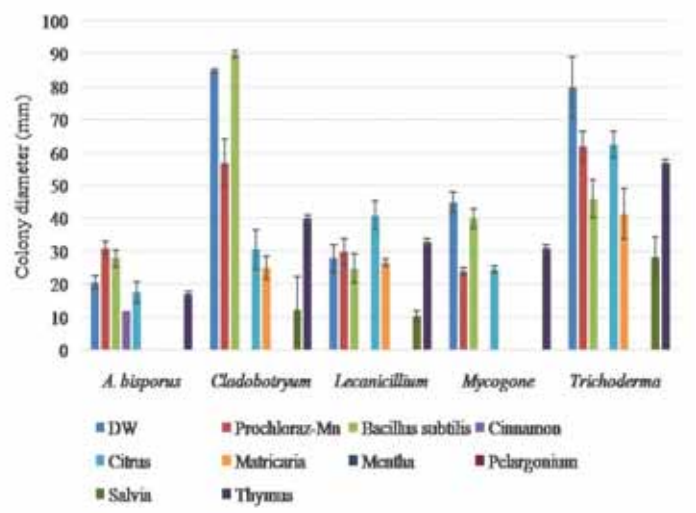

Figure 2. Influence of volatile fraction of essential oils on mycelial colony diameter

Based on the results it can be concluded that the selectivity of essential oils is not satisfying. Those oils that do not have a inhibiting effect on button mushroom mycelium do not inhibit the pathogens growth as well. The essential oils (cinnamon, mentha, geranium) with significant inhibition on pathogens block button mushroom growth too. The bacterium *B. subtilis* only affected *Trichoderma*, but the contact effect is acceptable.

Results of test on mushroom fruit bodies

Fig. 3 and 4 show the effect of essential oils in the fruitbodies. As it can be seen on Fig. 3, each undiluted oil cause similar deep, dark lesions. Essential oils at this concentration damage fruit bodies so much that they cannot be used or consumed anymore. The undiluted bacterial product caused brown lesions, while the active agent (prochloraz-Mn) of a licensed product caused lighter, but distinctive lesions on the mushrooms.

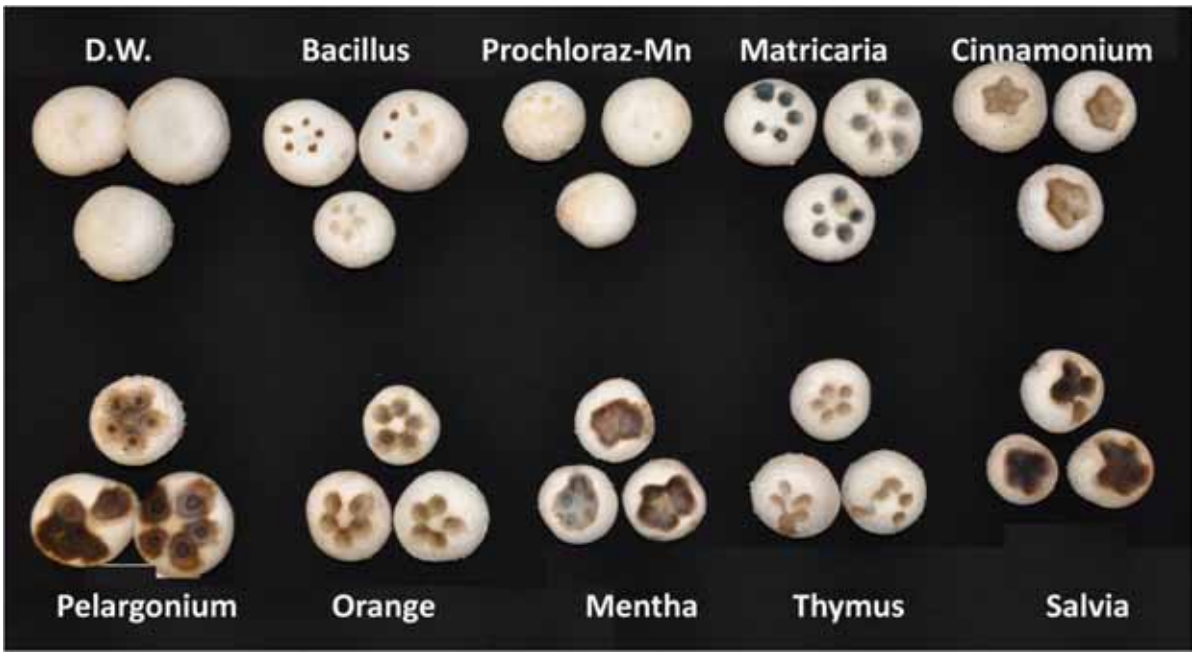

Figure 3. Effect of concentrated essential oils and active substances on to mushroom fruit bodies after 24 hour incubation

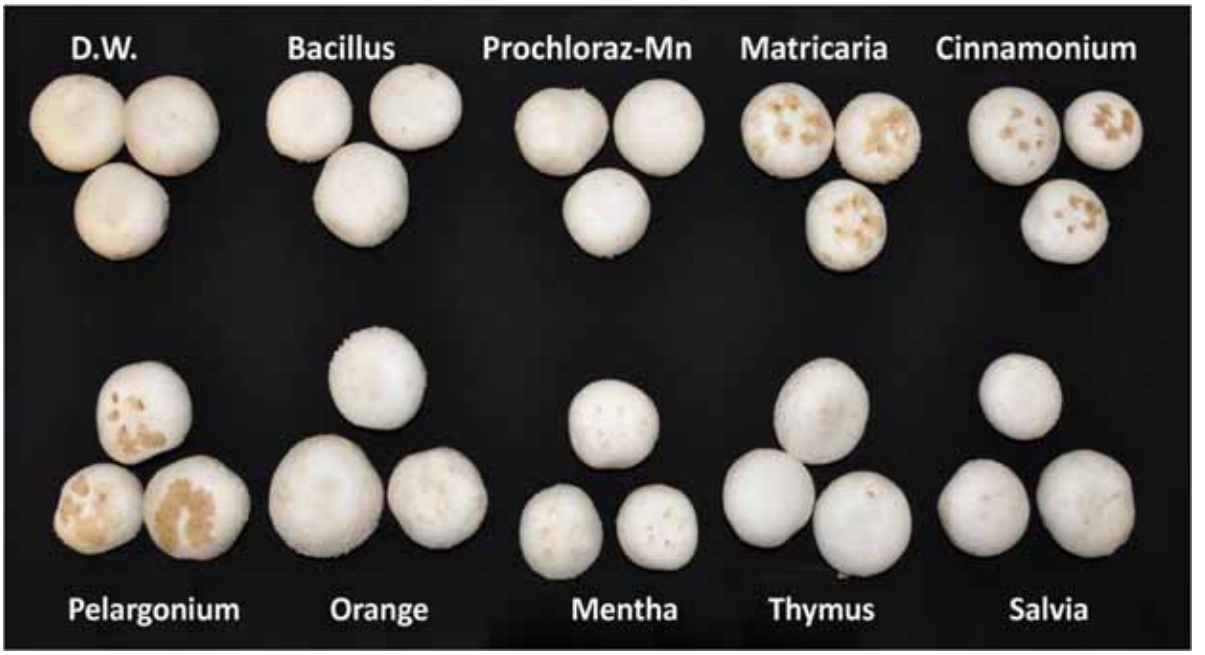

Figure 4. Effect of diluted essential oils (1000×) and active substances onto mushroom fruit bodies after 24 hour incubation

Fig. 4 shows the results of the tests conducted with 1000 times diluted oils. At this concentration spots were recorded in case of cinnamon, chamomile and geranium. The lesions caused by spearmint and sage oils were smaller and not as deep. The diluted bacteria product did not induce any spots, thus from this point of view it can be applied during the growing period.

CONCLUSION

A number of studies have examined the effect of essential oils on the pathogens of button mushroom. In some cases, the positive effects were proven and even the components responsible for inhibition were identified. In this study 7 essential oils were included, on which previously no or not complete tests have been conducted. Results of the *in vitro* tests show that cinnamon, geranium and spearmint oils have a strong inhibiting effect on the pathogens *Cladobotryum*, *Mycogone*, *Lecanicillium* and *Trichoderma.* It has been proven that essential oils not only have a contact effect, but also by vaporizing, the active agents can block the vegetative growth of pathogens. Some previous studies [8, 9] have not included button mushroom mycelia in the tested species. Our experiment shows that since essential oils are not selective enough and damage the mycelia and the fruit body of the cultivated mushroom as well, further *in vivo* studies of the above mentioned three species are recommended. Although a certain level of yield loss is predictable, it should be noted that already licensed and applied products have the same effect too. Since essential oils proved to be fungistatic, alternative application method (e.g. preventive treatment) should be considered. Based on the price and difficulty in application (rooted in the non water soluble property of oils), essential oils probably will not be competitive with traditional disinfectant products.

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REFERENCES

[1] Fletcher JT and Gaze RH. (2008). Mushroom Pest and Disease Control, a ColourHandbook. Manson Publishing, Boston.

[2] Romaine CP *et al.* (2008). Emergence of benzimidazole-resistant green mold, *Trichoderma aggressivum*, on cultivated *Agaricus bisporus* in North America. *Mushr. Sci.* 17: 510-523.

[3] Bonnen AM and Hopkins C. (1997). Fungicide resistance and population variation in *Verticillium fungicola*, a pathogen of the button mushroom, *Agaricus bisporus*. *Mycol. Res.* 1: 89-96.

[4] Bakkali F *et al.* (2008). Biological effects of essential oils - A review. *Food Chem. Toxicol*. 2: 446-475.

[5] Shah S and Nasreen S. (2011). Evaluation of Bioagents against the Infection of Green Mould (*Trichoderma* spp.) in *Pleurotus sajor-caju* Cultivation. *Intl. J. of Plant Pathol.* 2: 81-88.

[6] Górski R *et al.* (2010). Effect of selected natural essential oils on *in vitro* development of fungus *Trichoderma harzianum* found in common mushroom (*Agaricus bisporus*) cultivation. *Ecol. Chem. Eng*. 17(2): 177-185.

[7] Sokovic MD *et al.* (2009). Chemical composition of essential oils of *Thymus* and *Mentha* species and their antifungal activities. *Molecules* 14: 238-249.

[8] Glamoclija J *et al.* (2008). Anti fungal activities of mint and thyme essential oils against mycopathogen *Mycogone perniciosa*. Abstract of the Sixth International Conference on Mushroom Biology and Mushroom Products. Bonn, Germany: 76.

[9] Potoènik I *et al.* (2010). Sensitivity of *Mycogone perniciosa*, pathogen of culinary-medicinal button mushroom *Agaricus bisporus* (J. Lge) imbach (*Agaricomycetideae*), to selected fungicides and essential oils. *Int. J. Med. Mushr*. 12(1): 91-98.

[10] Regnier T and Combrinck S. (2010). *In vitro* and *in vivo* screening of essential oils forthe control of wet bubble disease of *Agaricus bisporus*. *S.Afr. J. of Bot.* 76(4): 681-685.

[11] Tanovic B *et al*. (2009). *In vitro* effect of essential oils from aromatic and medicinal plants on mushroom pathogens: *Verticillium fungicola* var. *fungicola*, *Mycogone perniciosa*, and *Cladobotryum* sp. *Arch. Biol. Sci*. 61(2): 231-237.

[12] Gao M *et al.* (2014). Browning inhibition and quality preservation of button mushroom (*Agaricus bisporus*) by essential oils fumigation treatment. *Food Chem*. 149: 107-113.