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Cultivation of three medicinal mushroom species on olive oil press cakes containing substrates

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ABSTRACT

Olive oil press cakes (OOPC) represent a waste that has a negative impact on environment. OOPC have little or no use and because of that solutions for their alternative use are sought after. In our experiments we investigated substrate mixtures composed of different proportions of OOPC, wheat bran, crushed corn seeds and beech sawdust for cultivation of *Ganoderma lucidum*, *Lentinula edodes* and *Grifola frondosa* fruiting bodies. The increasing amount of OOPC in fruiting bodies cultivation substrates resulted in decreasing production of fruiting bodies. Results show, that although OOPC in small portion can be successfully used as a medicinal mushroom fruiting bodies cultivating substrate, their use is rational only, if no other substrate composing materials can be found or when OOPC usage solves the problem of its deposition.

Key words: *Ganoderma lucidum, Grifola frondosa, Lentinula edodes*, mushroom cultivation, olive oil press cakes

IZVLEČEK

GOJENJE TREH VRST MEDICINSKIH GOB NA SUBSTRATIH VSEBUJOČIH OLJČNE TROPINE

Oljčne tropine (OT) predstavljajo odpadek s škodljivim vplivom na okolje in omejenimi možnostmi uporabe. OT imajo malo ali nobene uporabne vrednosti, zaradi česar se išče načine za njihovo alternativno uporabo. V naših poizkusih smo preizkušali substrate iz različnih deležev OT, pšeničnih otrobov, zdrobljenega koruznega zrnja in bukove žagovine za gojenje trosnjakov gliv *Ganoderma lucidum*, *Lentinula edodes* in *Grifola frondosa*. S povečevanjem deleža OT v substratu smo opazili trend zmanjševanja biološke učinkovitosti obroda. Rezultati kažejo, da čeprav lahko OT v manjših deležih uspešno uporabimo za substrat za gojenje trosnjakov, je to smotrno le v primerih, ko ni na voljo drugih primernejših sestavin substrata ali takrat, ko je dodatek OT namenjem preprečavanju negativnih vplivov teh odpadkov na okolje.

Ključne besede: *Ganoderma lucidum, Grifola frondosa, Lentinula edodes*, gojenje gob, oljčne tropine

1 INTRODUCTION

Olive oil press cakes (OOPC) represent a waste with a great negative impact on environment in Mediterranean countries, where many olive oil producing plants are located. OOPC have little or no use and solutions for their alternative use are sought after. It is well known that mushrooms can be cultivated on broad assortment of organic matter, including sawdust, straw, weeds, husks, compost and others. There were also reports of successful OOPC usage as a mushroom cultivating media (Soler-Rivas et al., 2006; Ruiz-Rodriguez et al., 2010; Zervakis et al., 2013).

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All experiments were performed at the mycology laboratory of MycoMedica d.o.o., Podkoren 72, 4280 Kranjska Gora, Slovenia

Ganoderma lucidum (Curtis) P. Karst., *Grifola frondosa* (Dicks.) Gray and *Lentinula edodes* (Berk.) Pegler are popular medicinal mushrooms with immune system strengthening, anticholesterolemic, antitumor, hepatoprotective, antidiabetic and other medicinal properties (Wasser, 2010; Powel, 2010). Successful cultivation of these species on OOPC containing substrates would have multiplicative positive effects, firstly on environment being polluted by OOPC and secondly on humans ingesting the cultivated fruiting bodies and medicinal substances they contain.

In our experiments we tested substrate mixtures composed of different portions of OOPC, wheat bran and beech sawdust for cultivation of *Ganoderma lucidum*, *Lentinula edodes* and *Grifola frondosa*. We aimed to analyze how medicinal mushroom cultivation substrates containing variable OOPC concentration influence the production of *G. lucidum*, *L. edodes* and *G. frondosa* fruiting bodies.

2 MATERIALS AND METHODS

Lentinula edodes strain No. 4080, *Ganoderma lucidum* strain Gal5 from culture collection of Zavod za naravoslovje (Institute for natural sciences), Ljubljana, and *Grifola frondosa* strain Gf3 from fungal collection of Wood Science and Technology department, Biotechnical Faculty, University of Ljubljana, Slovenia were used. Cultures were maintained on potato dextrose agar (Difco) at 24 ºC.

Substrates were composed of variable proportions of OOPC (Torklja, Koper, Slovenia), beech sawdust (BS) (Gorazd Rant s.p., Železniki, Slovenia), wheat bran (WB) (Mlin Katić, Velika vas pri Krškem, Slovenia) and gypsum (Rigips Austria GmbH, Saint-Gobain, Austria) (Table 1). Substrate components were mixed and water content adjusted to 65 %. Substrate was filled into polypropylene bags with breathing filters (3.5 kg for *L. edodes* and *G. lucidum* and 3.0 kg for *G. frondosa*) and sterilized for five hours at 121 ºC. At least four replicates were prepared for each substrate mixture.

Table 1. Substrate mixtures used for *Lentinula edodes, Grifola frondosa* and *Ganoderma lucidum* cultivation

Olive oil press cakes (OOPC) (%)	Wheat bran (WB) (%)	Beech sawdust (BS) (%)	Gypsum (%)
80	18	0	2
60	18	20	2
40	18	40	2
20	18	60	2
0	18	80	2

After the sterilization and cooling process substrates were inoculated with 100 g of *Ganoderma lucidum*, *Lentinula edodes* or *Grifola frondosa* mycelium, cultivated on rye grains, mixed by hand and incubated at 24 ± 1 ºC in a dark growth chamber. When the surface of the overgrown substrate became dark brown (*L. edodes*) or when primordia started to form (*G. frondosa* and *G. lucidum*), bags were moved into cultivation room with 17 ± 2 ºC, 10 hours of light daily and 80 % relative humidity. Fruiting bodies were harvested after they fully matured and their fresh weight determined. Biological efficiency (BE), being fresh fruiting bodies weight divided by weight of fresh substrate, multiplied by 100, was calculated (Royse and Sanchez-Vasquez, 2003). Because *G. lucidum* fruiting bodies are usually used and sold in dry form, BE for this species was calculated using dry weight of fruiting bodies after drying at 60 ºC for 48 hours (to constant weight).

All experiments were conducted in a mycological laboratory of MycoMedica d.o.o. company, Podkoren (Slovenia).

3 RESULTS

Lentinula edodes mycelium tends to grow slower and in some cases ceases to grow, if the substrates contained 80 % OOPC (Figure 1). Also there was a negative impact of OOPC on the growth of *L. edodes* mycelia and also its maturation. Substrates containing higher proportions of OOPC had a tendency to mature (change of color) later than substrates with lower OOPC share (Figure 1).

Figure 1. Substrates inoculated with *Lentinula edodes* mycelia containing (from left to right column) 80 %, 60 %, 40 %, 20 % or 0 % olive oil press cakes (OOPC).

Highest BE (38 %) of *L. edodes* fruiting bodies was calculated on substrates composed of 0 % OOPC, 80 % BS, 2 % gypsum and 18 % WB. Biological efficiency of fruiting bodies decreases in correlation to increasing proportions of OOPC in the growing substrates. When substrate contained 80 % OOPC, fruiting bodies ceased forming completely (Figure 1).

Figure 2. Deformed *Ganoderma lucidum* fruiting bodies emerging from substrate containing 80 % olive oil press cakes.

Figure 3. Non-deformed *Ganoderma lucidum* fruiting bodies emerging from substrate containing without olive oil press cakes.

Biological efficiency of *Grifola frondosa* fruiting bodies was reduced with increasing share of OOPC in the cultivating substrate. With *G. frondosa* higher fruiting bodies yields (62 %) were obtained on substrates not containing OOPC (Figure 4).

Ganoderma lucidum fruiting bodies yields also decreased with increasing portions of OOPC in the growth substrate (Figure 4). At higher OOPC content (60 % and 80 %) in the substrate fruiting bodies tended to have slight deformations in the shape (Figure 2), and were more sensitive to mold and bacterial infections. Development of *G. lucidum* fruiting bodies on 0 % OOPC containing substrates was not hindered (Figure 3).

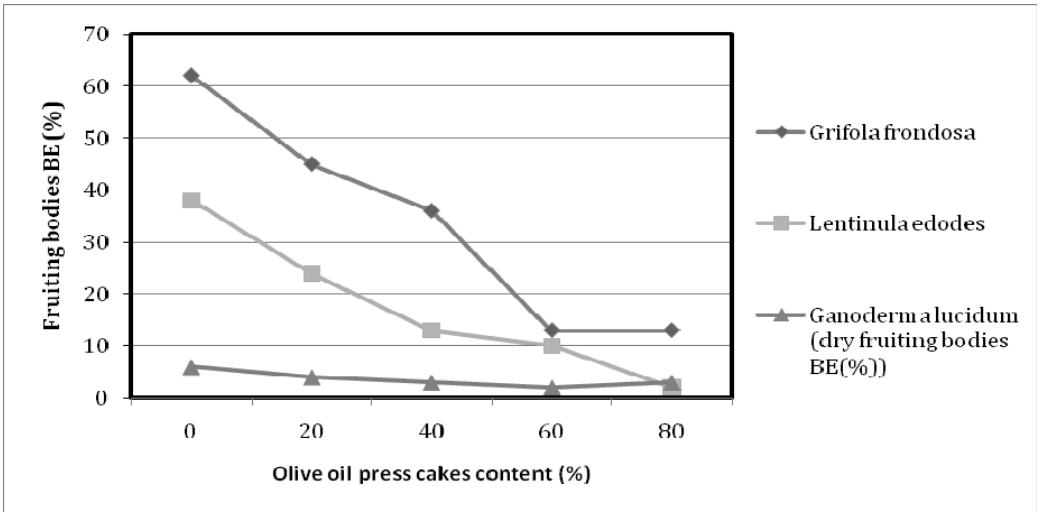

Figure 4. Biological efficiency (BE (%)) of *Lentinula edodes, Ganoderma lucidum* and *Grifola frondosa* fruiting bodies cultivated on olive oil press cakes containing substrates. (BE (%) of *Ganoderma lucidum* was calculated for dry fruiting bodies.)

4 DISCUSSION

Higher proportions of OOPC contained in cultivation substrates hindered the formation of *Lentinula edodes, Ganoderma lucidum* and *Grifola frondosa* fruiting bodies (Figure 4) and as well caused their deformation (Figure 2). With substrates not containing OOPC no yield reduction and fruiting bodies deformation was noticed (Figure 3). With *L. edodes* hindered mycelial growth was noticed immediatelly after inoculation (when mycelia ceased to grow completely), and during the substrate incubation period (when mycelia was maturing slower) compared with other substrates (Figure 1).

Hindered mycelia growth during incubation period as well as decrease of fruiting bodies yield on OOPC containing substrates could be the consequence of polyphenols contained in OOPC (Lakhtar et al., 2010; Zervakis et al. 2013). Beside polyphenolic compounds, a low porosity of OOPC and consequently lower substrate aeration and low water retaining capacity could be the reason for slower mycelial growth and lowered yields of fruiting bodies. It was found out that aeration greatly influences mycelial overgrowth and *L. edodes* fruiting bodies yields (Kalberer, 1995; Donoghue and Deninson, 1995). On the other hand fungal species and strains ability to utilize OOPC, or exposition to higher content of polyphenolic compounds in the substrate could have a significant influence on fruiting body yields. Strain characteristics tend to strongly influence mycelial growth as well as quantity and quality of produced fruiting bodies (Diehle and Royse, 1986; Royse and Bahler, 1986). Reduction of fruiting bodies yields on higher proportions of OOPC containing substrates is in accordance to the findings of other authors, who tested OOPC as a substrate component for cultivation of *Pleurotus ostreatus* (Ruiz-Rodriguez et al., 2010), *Pleurotus pulmonarius* (Soler-Rivas et al., 2006) as well as other *Pleurotus* and *Agrocybe cylindracea* species (Zervakis et al., 2013).

Nevertheless, the results show that OOPC in small proportions can be successfully used as a supplement to the medicinal mushrooms cultivating substrate. This application is reasonable only, if no other substrate composing materials are available, or when OOPC usage solves the problem of its deposition. Zervakis and coworkers (2013) found that composting of olive mill waste greatly increases the BE of produced fruiting bodies. This method could be used also with OOPC, potentially reducing its negative effect on mycelium growth and mushroom yields.

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