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MUSHROOM MYCELIA CULTIVATION ON DIFFERENT AGRICULTURAL WASTE SUBSTRATES

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Abstract

Increasing demand for edible or medicinal mushrooms has led to investigation into the suitability of sawdust and agricultural wastes as substrates for commercial production. Effective use of bio-resources by waste-free processing and production of nutraceuticals or ingredients for functional foods are the main directions in biotechnology. The aim of this work was to evaluate the growth capacity of mushroom mycelia on substrates of sawdust or agricultural wastes mixed with different amendments. Four variants of agricultural substrates (wheat and sorghum each of them mixed with CaSO4 and dolomite amendments) and seven mushroom species (Flammulina velutipes, Laetiporus sulphureus, Ganoderma lucidum, Ganoderma applanatum, Hericium coralloides, Trametes versicolor and Lepista nuda) were used. The mycelial cultures were initially grown in Petri dishes on 2 % malt extract agar or PDA media at 25 ^{0}C in the dark. After one week, the mycelium of each mushroom species was transferred to different sterilized grain, wheat, sorghum or barley straws or sawdust as substrates with various amendments. Various degrees of grain coverage with mycelia depending on substrate and mushroom species were obtained. The results obtained give the possibility of high quality inoculum using cheap renewable resources and the future extension of the research at the mushroom farm for evaluating the effectiveness of this inoculum for fruiting bodies obtaining. Better utilization of these recyclable materials by mushroom cultivation releases important land surfaces and also eliminates the polluting factors from the terrestrial ecosystems. After finishing the culture cycle, the spent substrate represents a valuable reusable resource as constituent material in nutritional mixtures for horticultural cultures, in bioremediation of some degraded soils or contaminated with various pollutants, of waste water, having a positive impact on improving the sorrounding environment.

Key words*: Mushrooms mycelia, agricultural wastes substrates, amendments, growth efficiency.*

INTRODUCTION

Following the agricultural sector activities very high quantities of lignocellulosic wastes result. These are represented by different parts of the cultivated plants (eg. wheat, sorghum, rice) that can be straws, stalks, husks.

Rani et al., 2008 show that the quantity of residual straws rises at 70 million tons per year in India. The European countries are also rich in wheat straws which is the cheapest crop residues in this part of the world (Ćilerdžić et al., 2014; Knežević et al., 2013). A quantity of wheat straw wastes is also available for Europe in some studies and is around 170 million tons (Knežević et al., 2013; Tabka et al, 2006). The problem of removing these wastes is also the subject of other studies around the world. According to some authors the solutions based on burning the sorghum stalks threat the health by respiratory diseases. The storage of the sorghum wastes on the fields can promote the occurence of the pests represented by insects and diseases that can sicken the crops (Rani et al., 2008). The wheat and rice straws are also eliminated by burning, producing in turn problems related to environmental pollution as Yang et al., 2013 show. Solving the problem of lignocellulosic agricultural wastes can be realised by promoting a biotechnological process that involves the use of the Basidiomycetes. Thereby the huge waste quantities coming from the cereal harvesting could constitute a valuable nutrient resource that with the help of a bioconversion technology could be recycled in useful products (Tan and Wahab, 1997).

The use of the Basidiomycetes as a biological conversion technology of the mentioned wastes resides in the mushroom ability to secrete the hydrolysis or oxidising enzymes. The mushrooms *Pleurotus* and *Lentinus* are two examples of species that own enzymes systems based on endoglucanase, laccase and phenoloxidases (Rani et al., 2008). *Trametes versicolor* is capable to solubilise lignocellulosic materials with the help of an enzymes mixture composed from peroxidases, celulases and glucose oxidases (Vyas et al, 1994). From literature, the *Pleurotus* mushrooms can adapt for growing and fruiting on a large variety of lignocellulosic wastes (Mikiashvili et al, 2006). Taking into account the use of mushrooms as potential straw degradation technology, basidiomycetes mycelium growing technologies on agricultural origin substrates can be investigated and designed. According to some sources the mushroom cultivated on the first place in the world is *Agaricus bisporus*, *Pleurotus ostreatus* being on the second place while on the third place is situated *Lentinula edodes*. Other mushrooms which follow the three most cultivated basidiomycetes worldwide are *Auricula auricula*, *Flammulina velutipes* and *Volvariella volvacea* (Rühl et al, 2008; Carmen Sanchez, 2004; Carmen Sanchez, 2009; Aida et al., 2009; Reis et al., 2011). The *Pleurotus* mushroom, known also as the oyster mushroom can be cultivated with excellent results under semi controlled conditions in a small space by using agricultural waste (Bhatti et al., 2007). Some sources indicate the fact that paddy straws are the best substrate for *Pleurotus* cultivation (Mane et al., 2007). Another important mushroom with increased higher demand namely *G. lucidum* has led to research in what concerns the suitability of sawdust and agricultural wastes as substrates for commercial bag cultivation of the mushroom. The cultivation on solid substrates has been declared as a success (Peksen et al., 2009). A study reveal that fruiting bodies of mushrooms including *G. lucidum* have been produced on solid cultures in a traditional manner using substrates such as grain, sawdust or wood (Peksen et al., 2009).

The aim of this work was to evaluate the growth capacity of mushroom mycelia on substrates of sawdust or agricultural wastes mixed with different amendments.

MATERIALS AND METHODS

Mushrooms culture

Seven mushroom species, such us: *Flammulina velutipes, Laetiporus sulphureus, Ganoderma lucidum, Ganoderma applanatum, Hericium coralloides, Trametes versicolor* and *Lepista nuda* were used for the experiments. The mushroom species were kindly provided from the Culture Collection of Mushrooms of the Faculty of Biotechnology- UASVM, Bucharest.

Growth conditions

Mycelial cultures were initially grown in Petri dishes (90 mm in diameter) on 2% malt extract agar or PDA (potato-dextose-agar) media at 25^0C in the dark. One week after, the mycelium of each mushroom species (agar segments of 10x10 mm) was transferred to different sterilized grain, wheat, sorghum or barley straws or sawdust as substrates with various amendments (Table 1).

Table 1. Variants of agricultural substrates

Variants	Substrates	Amendments
V1	Wheat – 420 g	$CaSO_4$ – 12,6g
V2	Wheat – 420 g	Dolomite – 12,6g
V3	Sorghum – 500 g	$CaSO_4$- 15 g
V4	Sorghum – 500 g	Dolomite – 15 g

After the inoculation the experimental samples were incubated at 25^0C at dark, for 15 days. After this period the growing ability of the fungi species mycelium an the degree of grain coverage with mycelium were evaluated.

RESULTS AND DISCUSSIONS

For the investigation of the growing ability of the fungi on different natural plant substrates, the mycelium of *Flammulina velutipes, Laetiporus sulphureus, Ganoderma lucidum, Ganoderma applanatum, Hericium coralloides, Trametes versicolor* and *Lepista nuda* was used for inoculating four types of experimental variants: V1 (wheat grains with $CaSO_4$) as amendment; V2 (wheat grains with dolomite) as amendment; V3 (sorghum grains with $CaSO_4$) and V4 (sorghum grains with dolomite). The dolomite is an amendment for soil for long- term, that conains calcium oxide and magnesium, having beneficial effects on the soil. Currently, the dolomite is used with modern soil cutivating technologies. After 3 – 4 weeks from the grains inoculating and incubation in the dark at approximately 24^0C it was found that that depending on the substrate nature, the covering degree of the substrate with mushroom mycelium was different. Thus, in the case V1 (wheat with $CaSO_4$) and V2 (wheat with dolomite) of experimental variants, mycelia of ***Ganoderma lucidum*** invaded the grains at a rate of 75% and 30% respectively (Figure 1). In what concerns the substrate variants V3 (sorghum with $CaSO_4$) and V4 (sorghum with dolomite), the covering degree of the grains with mushroom mycelium was 90% and 100% respectively (Figure 1). Following these experiments it was found that the optimal substrates for mycelium development in the first 4 weeks following the inoculation, where the ones consisting of sorghum grains with different amendments. After 8 weeks of de culture on tested substrates, on variant V1 (wheat with $CaSO_4$) even fruiting bodies developed (although initially the invasion of grains by the mushroom mycelium was more slowly) (Figure 2).

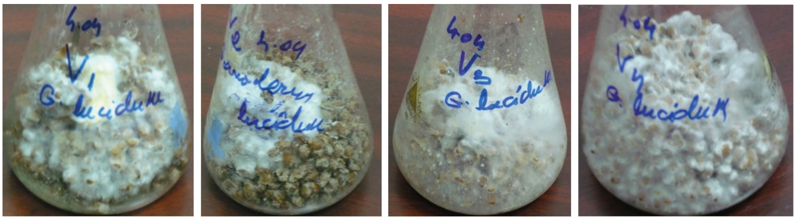

Figure 1. Different degrees of grain coverage with *Ganodema lucidum* mycelium depending on substrate, four weeks after. Figure 2. Fruiting bodies developed after eight weeks of culture on V1

In what concerns the ***Ganoderma applanatum*** species, the mycelium of this mushroom has optimally developed on the substrate represented by wheat with $CaSO_4$. The degree of coverage being 100%. The wheat covered with *G. applanatum* mycelium was used to inoculate a wheat straw substrate. After 8 weeks on this type of substrate, in which the mushroom invaded completely the straws, fruiting bodies primordiums developed (Figure 3).

Figure 3. Fruiting bodies of *G. applanatum* developed on sawdust, eight weeks after

Flammulina velutipes is known for being edible and also considering the existing information in the literature (Bao et al., 2009) it has medicinal properties, highlightening immunomodulatory, antitumoral and antioxidant properties. After 6 weeks from *F. velutipes* mycelium grain inoculating it has been found that the covering degree of the substrate was 100% on all the tested variants. On the variants V3 (sorghum with $CaSO_4$) and V4 (sorghum with dolomite) fruiting bodies of this mushroom have appeared (Figure 4). The wheat covered with mycelium was used to inoculate another wheat straws substrate with 3% $CaSO_4$. After 8 weeks on this type of substrate, in which the mushroom invaded completely the straws but without making fruiting bodies.

Figure 4. *Flammulina velutipes* after six weeks on grain substrates mixed with different amendments

In what concerns the ***Laetiporus sulphureus*** species, the mycelium of this mushroom has abundant developed on all the tested substrate variants, except on the substrate variant V4, represented by sorghum with dolomite (Figure 5). The mycelium appearance was dusty and the colour of the mycelium turned from white in the first stage to intense beige to the end of culture period.

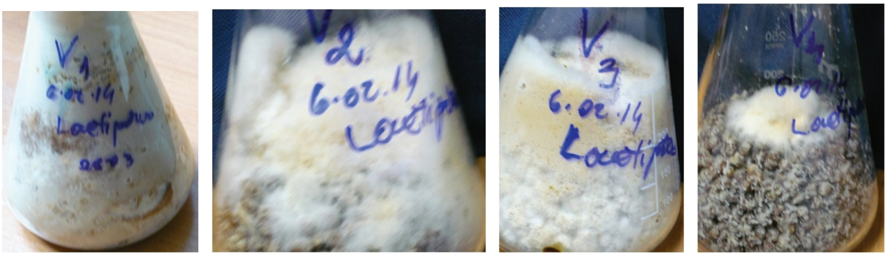

Figure 5. Mycelium of *Laetiporus sulphureus* developed on various substrates

Lepista nuda (*Clitocybe nuda*) is an edible mycorrhizal species, isolated from the ground level, around trees. It distinguishes by the production of a violaceous pigment at the mycelium level (the pigment was initially observed at the carphopores lamellae level). Researches performed on this species revealed antioxidant, antimicrobial properties (Dulger et al., 2002) and immunological effects (Lin et al., 2011). The fungal mycelium has abundant developed only on the variants V1 and V4 (Figure 6).

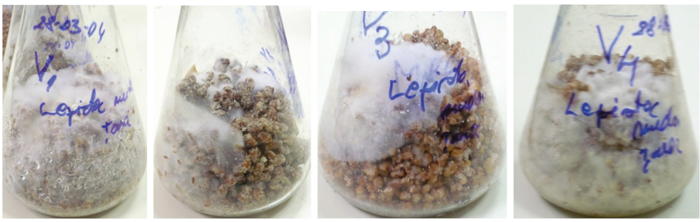

Figure 6. Different degrees of grain coverage with *Lepista nuda* mycelium depending on substrate, four weeks after

Trametes versicolor grows on decaying tree trunks. It has antitumor, hepatoprotective, antiviral, etc (Roupas et al., 2012; De Silva et al., 2013). On all the tested substrates, the degree of covering was 100 %. On wheat straws substrate after 8 weeks the mushroom mycelium invaded completely the straws developing fruiting bodies primordiums (Figure 7).

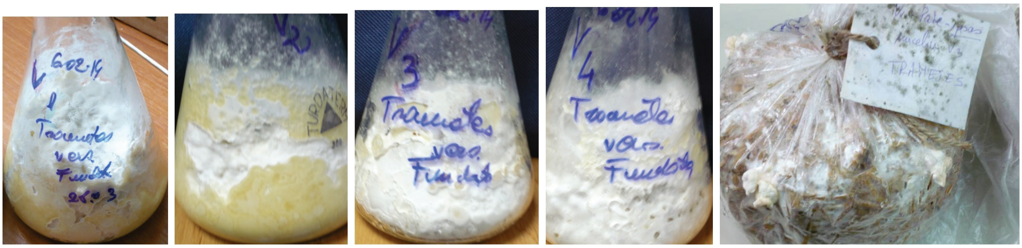

Figure 7. *Trametes versicolor* mycelium developed on wheat and sorghum substrates and fruiting bodies developed from inoculated straw

Hericium coralloides grows on dead hardwood trees. When young, the fungus is soft and edible. *Hericium* has been used as a traditional medicine in China and Japan for many years; but its medicinal uses are still being explored.

Hericium coralloides mycelium developed on all substrate variants investigated. After eight weeks, fruiting bodies were developed on V1 and V3 grain substrate (Figure 8).

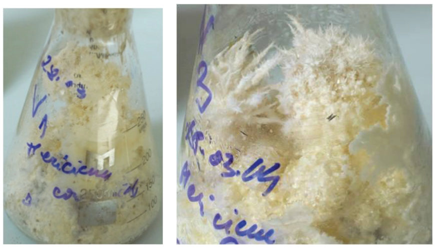

Figure 8. *Hericium coralloides* fruiting bodies developed on V1 and V3 grain substrate

CONCLUSIONS

Several variants with natural plant substrates with different amendments, for optimal substrate evaluation for mycelium growth in order to obtain fungal inoculum were carried out. The mushroom cultivation on different substrates reveals that using agricultural wastes mixed with different amendments, in the most cases, supported a good mycelial growth. Different degrees of grain coverage with mycelium depending on substrate and mushroom species. These results suggests the possibility of high quality fungal inoculum using a series of cheap renewable resources and the future extension of the research at the mushroom farm for evaluating the effectiveness of this inoculum for fruiting bodies obtaining. Better utilization of these recyclable materials by mushroom cultivation releases important land surfaces used for their storage and also eliminates the polluting factors from the terrestrial ecosystems. Furthermore, after finishing the culture cycle, the spent substrate represents a valuable reusable resource as constituent material in nutritional mixtures for horticultural cultures, in bioremediation of some degraded soils or contaminated with various polutants, of waste water, having a positive impact on improving the sorrounding environment.

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