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STUDY OF THE PROCESS OF SOLID-PHASE CULTIVATION OF HIGHER FUNGI ON MILLED SUNFLOWER SEEDS HULLS FOR THE OBTAINING OF COMPOSITE MATERIALS

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Currently, there is a great interest in the research of basidial fungi as ligninolytic enzymes producers capable of plant waste degradation. These properties are promising in composite materials creation, which can be employed as bio-packaging, bioplastics, and building materials. The purpose of the work was to study the possibility of using sunflower seed hulls for the cultivation of lignin-destroying fungi for the further development of composite biodegradable materials based on the obtained products. For that point, *Trametes polyzona* (Pers.) Justo (2011) and *Trichaptum abietinum* (Dicks.) Ryvarden (1972) growth on solid nutrient media based on sunflower seeds hulls were explored. It was shown that the fungi cultivation duration of the *Trametes polyzona* is 10 days and of the *Trichaptum abietinum* KS10 is 7 days. The suitability of the obtained products for use in the composition of biodegradable composite materials was assessed visually. The most durable structure of the packaging composite was obtained on nutrient media based on milled sunflower seeds hulls with the oat bran addition.

Keywords: Sunflower seeds hulls, packaging composite, basidiomycetes, *Trametes polyzona,* · *Trichaptum abietinum.*

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Introduction

Recycling secondary raw materials is one of the important areas of biotechnology. Wastes from the wood processing industry and agriculture, such as sawdust, husks, and bran, are often used as substrates for growing higher mushrooms. This raw material is rich in such compounds as lignin, cellulose, easily assimilated carbon, nitrogen, which are nutrition sources for basidiomycetes. For example, in industry, shiitake and maitake fungi cultivation is realized on straw, rice husks, oak, and maple wood [1–3].

One of the promising industry wastes for such employment is sunflower seeds hulls. World's sunflower total production is about 47 million tons. Sunflower seed husk is a by-product of the sunflower oil industry and represents 45–60% of the seed weight [4]. The main compounds of sunflower husk are cellulose up to 60%, pentosan up to 30% and lignin up to 30%. It also contains 3% fat, 3.4% protein, up to 3% ash, 1.4% phytomelan pigment [5]. This type of waste is rather difficult to utilize. It does not environmentally acceptable to burn sunflower seed husk in the field. While it direct soil introduction may present risks of plant contamination by pathogens [6]. Presently, sunflower seeds hull is most commonly used for animal feed production [4]. However, there are several developments for its application [4, 6–10]. Some authors recommend it as a biofuel for buildings heating [4]. Others [6] present the way to produce on its base the composts for soil improvement. As well, there is research shows that sunflower seeds hulls could be used as an additive in plastics, even for food packing production, after temperature processing [7]. However, more often, researchers offer to use it as a nutrition medium for basidiomycetes growth. Such as *Agaricus bisporus, Agaricus brasiliensis, Lentinula edodes, Pleurotus ostreatus, Schizophyllum commune, Grifola gargal, Grifola sordulenta, Grifola frondosa., Hericium erinaceus, Ganoderma lucidum, Hypsizygus marmoreus* [8–10].

The ability of basidiomycetes to destroy lignocellulosic raw materials is due to the presence of extracellular oxidative enzymes, for example, laccases, cellulases, pectinases, xylanases, phenoloxidases, etc. [11–14]. Today, there is a growing interest in the study of the biodegradation process of cellulose and lignin-containing substrates

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by wood-destroying fungi, in connection with the possibility of using the products of these biotechnologies in various fields of industry. However, it is important to choose cheap, affordable, and safe objects for this purpose.

Fungi of the genus *Trametes (Coriolus)* are known for their enzymes, especially laccases, which exhibit high biological activity. Their ligninolytic complex consists of enzymes such as laccases, lignin peroxidase, and manganese peroxidase [15–17]. Therefore, fungi of the genus *Trametes (Coriolus)* are promising for plant waste destruction. In the article [18], was shown that the fungus *T. versicolor* cultivation on a medium consisting of vinasse and cotton gin wastes leads to the highest yield of laccase with an activity of 1587 U/L. Also in [19], the possibility of *T. versicolor* cultivation on wheat bran with the addition of corn straw, corn cobs, rice straw, sawdust, and bagasse was studied. The maximum activity of laccase was achieved on a nutrient medium with wheat bran and corn straw and amounted to 32.09 U/g [19]. Another example is a study in which this higher fungus was grown on corn straw in a bioreactor. In this case, lignin degradation achieves up to 71% [20].

Recently, it has become relevant to create biomaterials based on wood processing industry waste and fungi mycelium, which can be used as bio packaging [21–23]. Mycelium is a fungi vegetative part with a high growth rate. Its hyphae are capable of penetrating free spaces and voids. Fungi mycelium could act as a "binder". For example, a bio carton was obtained using the mycelium of three types of fungi (*Ganoderma lucidum, Pleutorus ostreatus* and *Auricularia polytricha*) and sawdust. These material samples have high adhesion strength, thermal stability, and low swelling rate within 48 hours [24]. In addition, the American company Ecovative produces a heat-resistant, fire-resistant, waterproof and biodegradable material based on fungi and agricultural waste. By its characteristics, this material is not inferior to polystyrene and expanded polystyrene. This suggests that the resulting material could replace plastic [25]. The materials presented in [26] confirm this. In this paper, a biocomposite material was developed based on a *Pleurotus ostreatus* fungus mycelium cultivated on a saw dust-coir pith substrate. This biocomposite and polystyrene comparative analysis also was done. The bending tests results showed that for mycelial composite 0.18 MPa was required, and 0.03 MPa for polystyrene deformation. In addition, the biocomposite material had higher compression modulus and compressive strength than polystyrene (of approximately 2 and 50 times higher, respectively).

The higher fungi *Trametes polyzona* and *Trichaptum abietinum* KS10 isolated from natural conditions (Fig. 1) are promising objects for the development of a biodegradable composite material. Currently, these species have not been applied in industry and are used only in ethnomedicine [27–29].

Thus, basidial fungi cultivation on lignocellulose wastes and composite materials creation on their basis, which can be used to obtain bio packaging materials, are biotechnology promising areas.

Materials and Methods

The aim of the work was to study the possibility of using sunflower seed hulls for the cultivation of lignin-destroying fungi for the further development of composite biodegradable materials as analogues of cardboard. For this, it was necessary to choose lignin-utilizing fungi, the composition and method of preparation of the nutrient medium, and the cultivation conditions.

a b

Fig. 1. The studied Basidiomycetes in nature: a) *Trametes polyzona,* b) *Trichaptum abietinum*

Two basidiomycetes *Trametes polyzona* (Pers.) Justo (2011) and *Trichaptum abietinum* (Dicks.) Ryvarden (1972) KS10, whose fruit bodies were collected in the mixed forests of the Republic of Tatarstan and the Republic of Mari El, were chosen as the objects of study in this work. These xylotrophs are notable for their high content of biologically active compounds and synthesis of extracellular oxidative enzymes complex. Isolation of pure cultures from fruit bodies was carried out according to well-known methods [27].

The pure culture was stored at a temperature of 4±2 °C in Petri dishes on agar oat decoction (*Trametes polyzona*) and glucose-potato agar (*Trichaptum abietinum*).

Sunflower seeds hulls were sterilized at 120 °C for 1.5 hours [30]. Part of the raw material was previously ground in a mill (Bosch TSM6A013B, Ljubljana, Slovenia) to a particle size of about 0.2–0.3 mm.

Fungi were cultivated on the following samples of sunflower seeds hulls: not milled, milled, and milled with the addition of 1% oat bran as a solid nutrient medium was employed in the experiments. The medium thickness equaled 2–3 mm, the moisture amounted from 75 to 80%. The fungi cultivation was carried out at a temperature of 27 °C and pH equal to 6.

To determine the fungi growth rate on the sunflower seeds hulls, the growth coefficient (GC) and the average daily growth rate (ADGR) were calculated according to the standard method [31, 32].

The packaging composite material from the nutrient medium with the fungi mycelium was obtained using a heat press (Freesub P0708, Jinhua, China) under a temperature of 150 °C. Received products applicability for use in biomaterials proceeding was assessed visually. Obtained material should have closest rheological characteristics to thick cardboard.

Results

The choice of oat bran as an additional source of microelements, starch and protein was based on previous studies of *Trametes polyzona* growth on agarized culture media. It was then shown that the fungus develops better on nutrient media with oat broth [33]. Analysis of solid-phase cultivation of *Trametes polyzona* on the milled sunflower seeds hulls and milled sunflower hulls with the addition of 1 % oat bran showed practically the same growth coefficients. Therefore, their addition in other concentrations was not studied. While this indicator calculated for cultivation on unmilled hulls is approximately 2 times less (Table 1).

Evaluation of the mycelium growth dynamics (Fig. 2) showed that *Trametes polyzona* grows effectively on all studied substrates. The maximum colony diameter was achieved on the 10th day of cultivation and equals 9.0 cm.

According to the data shown in Figure 3, it can be concluded that the packaging composite materials production is possible on milled sunflower hulls and milled sunflower hulls with oat bran since their structure is dense and more similar to thick cardboard. The composite based on not milled sunflower seeds hulls is fragile and unformed.

Similar indicators were determined for the *Trichaptum abietinum* KS10 basidiomycete (Table 2). High growth rates were observed on a nutrient medium containing milled sunflower seeds hulls and oat bran, as well as on unmilled sunflower seeds hulls. However, in this case, the minimum value of the growth factor showed cultivation on the milled sunflower hulls.

The maximum diameter of the fungus colony of 9.0 cm was observed on the test media on the 7th day of cultivation. Although, during cultivation on a milled medium, this value was set only by the 10th day (Fig. 4).

Milled sunflower seeds hulls could be employed as the basis of the packaging material. As the received composite material, after *Trichaptum abietinum* KS10 cultivation on it has a satisfactory, similar to thick cardboard structure. While a sample obtained using unmilled hulls has a loose and fractional structure (Fig. 5).

The obtained results indicate the possibility of sunflower seeds hulls utilization with the use of *Trametes polyzona* and *Trichaptum abietinum* KS10 to obtain biomaterials.

Table 1. The results of growth parameters determine for the *Trametes polyzona* mycelium, cultivated on sunflower seeds hulls (n=3)

Nutrient medium	GC	ADGR, mm/day	Cultivation duration, day
Unmilled sunflower seeds hulls	22.90±0.46	3.82±0.08	10
Milled sunflower seeds hulls	47.20±1.39	3.85±0.13	
Milled sunflower seeds hulls with the addition of 1% oat bran	48.20±0.35	4.02±0.03	

GC – growth coefficient; ADGR – average daily growth rate

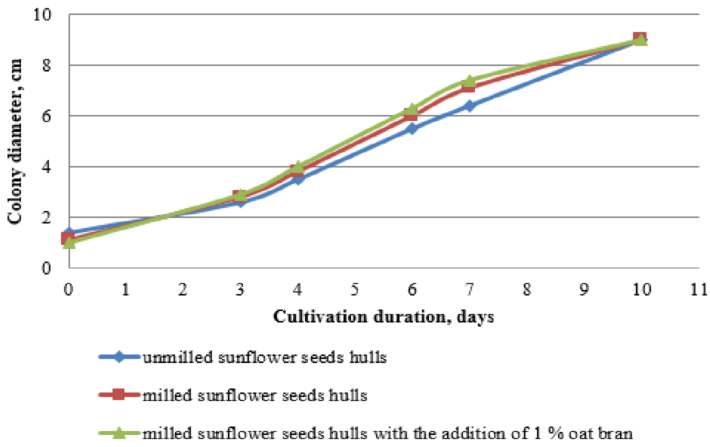

Fig. 2. Growth dynamics of the *Trametes polyzona* fungi cultivated on sunflower seeds hulls

Fig. 3. The composite materials obtained on the 6th day of *Trametes polyzona* cultivation on: a) unmilled sunflower seeds hulls; b) milled sunflower seeds hulls; c) milled sunflower seeds hulls with the addition of 1% oat bran

Table 2. The results of growth parameters determine for the *Trichaptum abietinum* KS10 mycelium, cultivated on sunflower seeds hulls (n=3)

Nutrient medium	GC	ADGR, mm/day	Cultivation duration, day
unmilled sunflower seeds hulls	61.71±0.86	5.14±0.07	7
milled sunflower seeds hulls	31.47±0.92	3.87±0.23	10
milled sunflower seeds hulls with the addition of 1% oat bran	94.29±1.48	5.24±0.08	7

GC – growth coefficient; ADGR – average daily growth rate

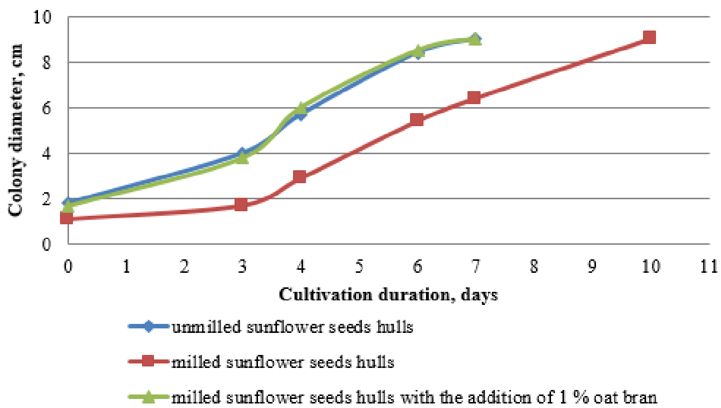

Fig. 4. Growth dynamics of the *Trichaptum abietinum* KS10 cultivated on sunflower seeds hulls

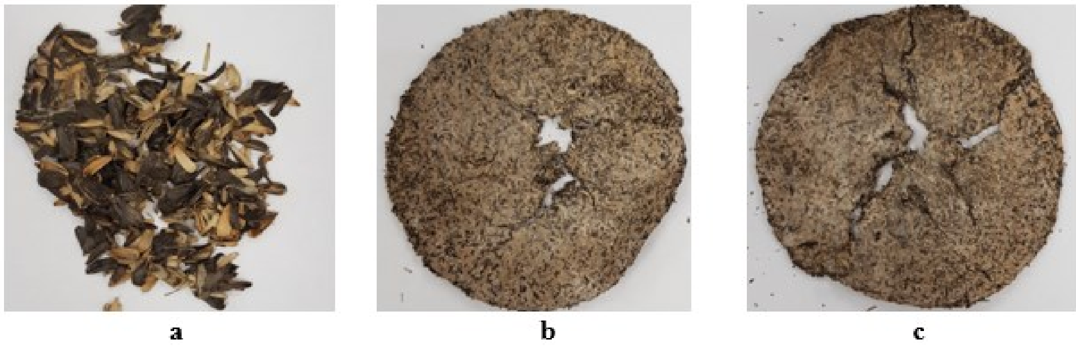

Fig. 5. The composite materials obtained on the 6th day of *Trichaptum abietinum* KS10 cultivation on: a) unmilled sunflower seeds hulls; b) milled sunflower seeds hulls; c) milled sunflower seeds hulls with the addition of 1% oat bran

Discussion

As a lignocellulosic raw material, was used sunflower seeds hulls, which are oil and fat industry waste. To create composite materials based on milled sunflower seeds hulls, two higher fungi with high ligninolytic properties were selected: *Trametes polyzona* and *Trichaptum abietinum* KS10.

For the cultivation of basidiomycetes, sunflower seeds hulls is not an optimal nutrient medium. For this substrate to meet the needs of the fungus, researchers are using different methods. Among them are: supplementation by Mn (II), Zn (II), Cu (II) salts the nutrient medium, glucose, urea, ammonium sulphate; milling a part or the whole substrate; composting nutrient media in different conditions; combining with other agricultural wastes [8–10]. For the development of technology for use in industry, simplicity, convenience, and availability of the materials, equipment, and methods used are important. Therefore, too great a variety of components added to the nutrient medium, complex and lengthy processing is most often not acceptable. Good growth rates were observed for fungi cultivation on sunflower seeds hulls with bran additive. Beyond that, with milled sunflower seeds husk introduction to the nutrient medium, the culture growth rate was increased significantly [9].

To determine how the milling of raw materials influence the fungi growth, their destructive ability, and the structure of the resulting packaging composite material, the following solid nutrient media were selected: 1) unmilled sunflower husk (particle size 4–13 mm), 2) crushed sunflower husk (particle size 0.2–0.3 mm), 3) crushed sunflower husk with the addition of 1% oat bran (particle size 0.2–0.3 mm). It is known that the nature of fungal mycelium growth is influenced by several factors: the cultivation temperature, the nutrient medium moisture, and pH value. The optimal conditions for growing these basidiomycetes are the temperature of 20 to 30 °C, the nutrient medium moisture of 60 to 80%, and pH of 5 to 6. As shown in [33], the optimal temperature for cultivating *Trametes polyzona* is 27 °C. In this regard, in this work for the cultivation of *Trametes polyzona* and *Trichaptum abietinum* KS10 the following conditions were chosen: temperature of 27 °C, the nutrient medium moisture of 72 to 80%, and pH of 6.

According to Tables 1 and 2, these basidiomycetes can be attributed to the fungi with an average growth rate. The *Trichaptum abietinum* KS10 fungus grows more intensively on all studied nutrient media. Both understudy fungi demonstrated the best growth results during cultivation on the milled sunflower seeds hulls with the addition of oat bran as an additional food source. Using this nutrient media, the growth coefficient of xylotroph *Trichaptum abietinum* KS10 fungus in comparison to the growth coefficient of *Trametes polyzona* was almost 2 times higher and equaled of 94.29±1.48 and 48.20±0.35, respectively. Additionally, cultivation time for the *Trichaptum abietinum*, compared to the *Trametes polyzona*, is reduced by 3 days. It can be assumed that oat bran, which is part of the nutrient medium, is the better growth stimulator, especially towards *Trichaptum abietinum* KS10. From the above, the use of *Trichaptum abietinum* KS10 is more effective.

To determine the possibility of employing investigated fungi in composite materials creation, the obtained solid media with their mycelium were exposed to mechanical and thermal actions. As can be seen from Figures 3 and 5, composite materials based on unmilled sunflower seeds hulls, after growing of studied fungi on it, had a fragile and undeveloped structure. However, milling the raw materials for further fungi cultivation leads to producing materials that are more durable. The obtained composite materials based on *Trametes polyzona* and *Trichaptum abietinum* KS10, grown on milled nutrient media, practically do not differ in their characteristics.

Thus, the possibility of growing *Trametes polyzona* and *Trichaptum abietinum* KS10 the higher fungi on sunflower seeds hulls and creating composite materials on their basis for further industrial use as bio packaging has been shown.

Conclusion

High growth rates of *Trametes polyzona* and *Trichaptum abietinum* KS10 on nutrient media containing sunflower seeds hulls were established. The maximum growth coefficient and average daily growth rate were achieved using the *Trichaptum abietinum* KS10 fungus on milled sunflower seeds hulls medium with the addition of 1% oat bran and amounted to 94.29±1.48 and 5.24±0.08 mm/day, respectively. The cultivation time is 7 days.

The prospect of using the *Trichaptum abietinum* KS10 and *Trametes polyzona* as a "binding agent" for the development of packaging materials based on milled sunflower seeds hulls with strong properties, similar to thick cardboard was shown.

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Conflict of Interest
The authors of this work declare that they have no conflicts of interest.

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