

# Propolis: An Enrichment Material for Mycelium Development of Oyster Mushroom (*Pleurotus ostreatus*)

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## Abstract

The effect of propolis, taken from honeybee hives in Tokat (Turkey), on mycelium development of oyster mushroom (*Pleurotus ostreatus*) was investigated. Propolis contains various chemical compounds and has antiviral, antibacterial and antifungal activities. Mushroom spores were inoculated in Potato Dextrose Agar (PDA) media containing different proportions of propolis. Mycelium development on media containing 0.10%, 0.30%, and 0.50% propolis occurred at the first, third, and fifth days, respectively. The fastest and most intense development was seen on medium containing 0.10% propolis. According to our results, propolis can be used as an enriching material in mushroom cultivating.

## Keywords

Propolis, *Pleurotus ostreatus*, Mycelium Development, Turkey

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## 1. Introduction

Mushrooms are very rich in amino acids, vitamins, and minerals; therefore they are very important in human nutrition and health [1]. Population growth and increasing food requirement in the world enhances the importance of natural products such as mushrooms. Thus, mushroom cultivation can be one of the most significant sectors in meeting food needs. Many species of mushrooms are grown in the world and demand for them is increasing steadily.

*Pleurotus* genus is found in *Pleurotaceae* family, *Agaricales* order, *Agaricomycetes* class and *Basidiomycota* phylum. Oyster mushroom (*Pleurotus ostreatus* (Fr. Kummer)), also known as “poplar mushroom” or “tree

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mushroom”, grows naturally on hardwood or decaying conifer, poplar and beech trees in tropical and temperate climates throughout the world [2] [3], but it is also commonly cultivated and among the most popular mushrooms. It is preferred due to its excellent flavor, high nutritional value, long shelf life and high biological efficiency [4]-[6].

Mycelia, found under the soil, are the main structures forming the mushroom and very important in mushroom cultivation [7]. During development, mycelia take mineral substances and water from the environment and form edible parts of the mushroom. Mycelium development is one of the most important factors that have a direct effect on productivity of mushroom cultivation. For this reason, enrichment of mycelial growth media as well as preventing it from contamination may help to improve productivity. Favorable effect of propolis on mycelium development of some mushrooms has been demonstrated by previous studies. For example Güler *et al.* [8] showed positive effect of propolis on mycelium development of *Morchella conica* and Güler *et al.* [9] reported that propolis directly increased mycelium development and product amount in *Agaricus bitorquis*.

Propolis is a kind of resin collected from plant shoots by honeybees which is then stored in the hive. It has antibacterial, antifungal, and antiviral effects [10] [11]. It contains many chemical compounds and is very rich in amino acids, aliphatic acids, aromatic acids, aromatic acid esters, aromatic aldehydes, flavons, ketons, and terpenoids [12] [13]. Propolis, due to its biological activities, may have an efficient role in preventing medium from contamination during mycelial growth. In this study, the effect of propolis on mycelium development of oyster mushroom *Pleurotus ostreatus*, was investigated.

## 2. Materials and Methods

### 2.1. Material

Mushrooms (*Pleurotus ostreatus*), produced in Agricultural Research and Application Center, Gaziosmanpaşa University, Tokat (Turkey) in 2014 were used in the study. Mushrooms were placed on a sterile paper, with gills opposite to direction of gravity, and completely covered with petri dishes. Spore prints were obtained after 12 to 24 hours. 0.10%, 0.30%, and 0.50% propolis extractions were added to Potato Dextrose Agar medium and mycelium development was followed.

### 2.2. Main Culture

Spores obtained from *Pleurotus ostreatus* fructifications were inoculated on PDA medium by multi-spore inoculation method. Primary mycelia grew after inoculation and homokaryon cultures were obtained. Agar discs were selected from the best grown 10 mycelia and transferred to petri dishes and then incubated for ten days at 27°C. Secondary mycelium development was observed and heterokaryon cultures were obtained [14]. Mycelium development was measured until they covered 3/4 of petri dishes. Developed mycelia were stored at +4°C.

### 2.3. Extraction Procedure

Propolis samples were taken from honeybee hives in Tokat. Propolis samples were ground and kept for 24 hours to obtain powder. Powdery propolis was weighed and ethyl alcohol was added with a ratio of 1:3 (g/ml), which is then stirred for five hours at 500 rpm by heating stirrer at +35°C. The solution was stored at +4°C for a month and during this period regularly stirred every day. At the end of this period, the solution was stirred for five hours by heating stirrer at +35°C once again. The solution was filtered by using Whatman 1 and Whatman 2 papers together in a funnel. This extraction was prepared twice. 1.5 ml of extract was taken by glass pipette and evaporated to dryness. The residue was heated to 80°C - 100°C for 20 minutes in 75 µl dry pyridine and 50 µl bis (trimethylsilyl) trifluoroacetamide (BSTFA) solutions (stock 1). 0.5 cc stock 1 was diluted by 10 cc pure ethyl alcohol (99%) and stock 2 was obtained. 0.10, 0.30 and 0.50 cc propolis extract was taken from stock 2 and mixed with 1000 cc distilled water, then 33.6 g PDA was added to each of the extracts. The mixture was sterilized at 121°C for 15 minutes and poured into sterile petri dishes, which formed the solid propolis added medium after cooling [15]-[18].

### 2.4. Mycelium Transfer

Mycelial agar discs (0.9 mm diameter) were centrally inoculated to media with propolis (0.10%, 0.30% and 0.50% proportions) and without propolis (control group). Mycelium development was observed and recorded for

20 days during incubation at 28°C (Figure 1).

## 2.5. Spawn Preparation

Spawn was obtained by placing mushroom culture onto steam-sterilized wheat grain (*Triticum aestivum* L.). For this purpose, 5 kg grain was boiled for 20 minutes, then filtered and partially dried (to a moisture of 75% - 80%). 25 g lime, to regulate pH of the medium, and 100 g plaster, to prevent wheat grains from sticking together, were added to the mixture. The mixture was transferred into 1 lt glass bottles up to 2/3 of its volume and sterilized at 121°C for 45 minutes. When wheat grain cooled, mycelial agar discs that were developed previously in PDA medium were added and allowed for incubation at 28°C. During incubation, bottles were shaken twice a day in order to prevent wheat grains sticking together. Mycelia obtained were stored at +4°C (Figure 2).

## 3. Results and Discussions

*Pleurotus ostreatus* is a popular mushroom species cultivated on various substrates and enriched media, such as agricultural by-products, weeds, and wastes [19] [20]. Having direct effect on productivity, mycelium development is a vital process in mushroom cultivation. Mushrooms get their food from the environment by means of mycelia. Mycelial growth rate, obtaining good quality spawns and product contamination is among important factors affecting productivity [21]. Propolis, as an enrichment material to growth media, may help to overcome such problems and improve productivity by promoting mycelial development as well as preventing it from contamination.

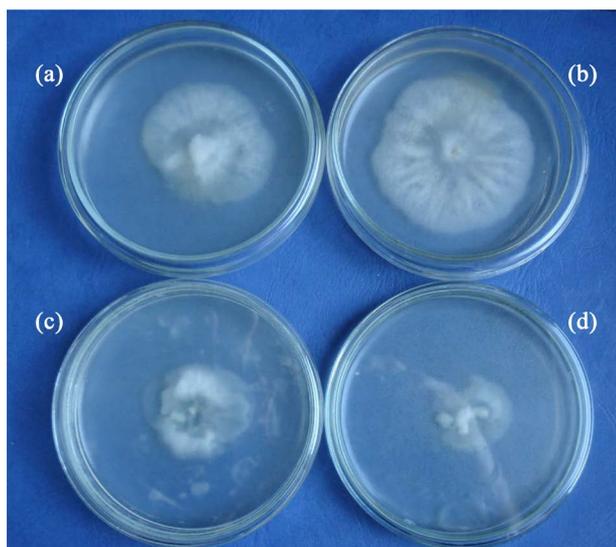


Figure 1. Mycelium development of groups (1 week). (a) Control; (b) 0.1 group; (c) 0.3 group; (d) 0.5 group.

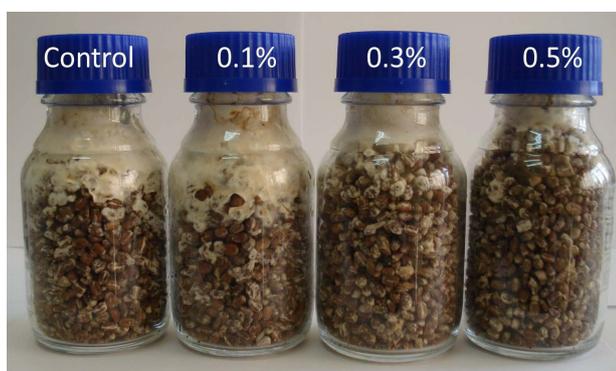


Figure 2. Spawn at different propolis concentrations.

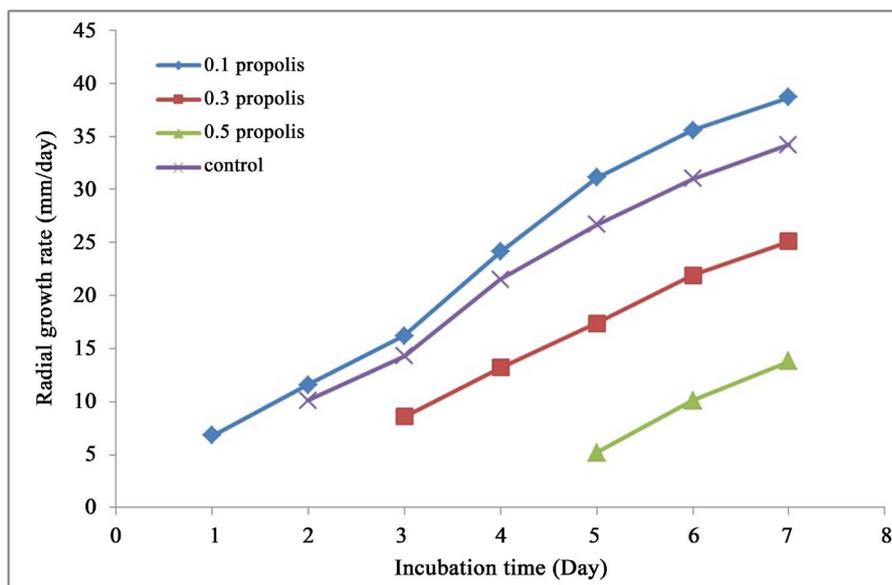


Figure 3. Mycelial development curves of groups.

In this study, we investigated mycelium development in PDA medium enriched with propolis. Mycelium development on PDA media containing 0.10%, 0.30% and 0.50% propolis occurred at the first, third and fifth days, respectively. Growth began at the second day in the propolis-free control medium. The fastest and most intense development was seen on medium containing 0.10% propolis (Figure 3). The results show that mycelia grew in a short time and increased the spawn quality in propolis added medium.

Our study showed that propolis may be thought as an additional nutrient in oyster mushroom mycelial growth media. Further research is needed to determine and compare nutritional value of mushrooms cultivated in propolis-enriched media with that of other media.

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