eISSN:2600-7920
INTI JOURNAL
Vol.1, 2018 (5)

# Alternative Substrate for *Hypsizygus tessellatus* Cultivation

[1]Thong Weng Hing, [1]Khu Yong Ming, [1*]Geetha Subramaniam

[1]Faculty of Health and Life Sciences, INTI International University
Persiaran Perdana BBN, Putra Nilai, 71800 Nilai, Negeri Sembilan, Malaysia

Corresponding Author: geetha.subramaniam@newinti.edu.my

## Abstract

The demand for mushrooms is increasing annually which opens an opportunity to developing countries to expand their mushroom industries as the mushroom culturing process converts agriculture waste to profit from crops. *Hypsizygus tessellatus* is a popular cultivated edible mushroom which is high in nutrient content and medicinal properties. This study was conducted to determine the effect of different parts of the stalk and different strength of potato dextrose agar (PDA) on the mycelium growth of *H. tessellatus* and to determine the effect of different alternative substrate, namely *Dicranopteris linearis* (fern leaf), dry leaf of *Terminalia catappa* and *Imperata cylindrica* (lalang grass) on the growth of shimeji mushroom, *H. tessellatus*. The mycelium from the upper and lower part of the mushroom stalk was cultured on half strength and full strength PDA, and the radius of the mycelium growth was measured. Mycelium from the lower part of the stalk had better growth effect. Full strength PDA also shown significantly better effects on mycelium growth. *H. tessellatus* were cultured on different types of substrate in capsule. Small pieces of *D. linearis*, *I. cylindrica* and dried leaf of *T. catappa* were found to be suitable alternative substrates for *H. tessellatus* cultivation where the mycelium growth in these substrate have no significant difference from the commercially used substrate which is mixture of saw dust and rice bran. The three alternative substrate mentioned above can be found in urban area in large amount which gave opportunity to people couldn't gain access to the commercial substrate chance to collect their own substrate for mushroom cultivation.

## Introduction

Mushroom is one of the most well-known fungi which is categorize in the class of Basidiomycetes and can be easily found growing on damp rotten wood trunk of trees in the forest or moist soil rich in organic substance (Rajapakse, Rubasingha & Dissanayake, 2007). Unlike plants that convert solar energy to chemical energy, mushroom like any other fungi, which does not contain chlorophyll utilize organic matters from plant, animal or other fungi as nutrition and energy source (Nasim et al., 2001). Edible mushroom can be easily found on the shelf of food market nowadays and are also popular in the list of the food menu as people love its special texture, flavour and aroma. It can be cooked as main ingredient or used as condiment for other dishes. Mushrooms are known to have high value of proteins, carbohydrates, multi vitamins, minerals, rich in folic acid (vitamin B) (Mat Amin, Azahar Harun, & Abdul Wahab, 2014), and most of the essential amino

acid (Mujić, Zeković, Lepojević, Vidović, & Živković, 2010). Mushroom could also prevent hypertension, high cholesterol and cancer (Mujić, Zeković et al., 2010) which increases its demand due to consumers having the awareness that consuming mushrooms are benefit to health. The Malaysia's National Argo-Food Policy (2011- 2020) has also listed mushroom as one of the high-value commodities due to its potential and promising high market demand. According to Mat Amin et al. (2014), the demand for mushrooms in the world is increasing and this will bring the opportunity for expanding the Malaysia mushroom industry as the climate of Malaysia is suitable for the cultivation of 17 varieties of edible mushroom.

According to Sanchez (2004), cultivation of edible mushroom is a biotechnology process, which aids in reducing and equally protecting the environment from excess solid waste. This has been an attractive point for developing countries to involve themselves in culturing mushrooms which converts agriculture waste to profit enable crops (Khan et al., 2012). Cultivation of mushroom using cheap substrate has also been studied in recent years as the process could convert waste product to nutritious food or therapeutic compounds (Tetiana & Victor, 2015). Many studies have reported the suitability of various substrates for mushroom cultivation including rice straw, maize, oak wood, horse chest nut, saw dust, cotton stalk and others (Zervakis, Philippoussis, Ioannidou, & Diamantopoulou, 2001). In Malaysia, sawdust and rice husk are commonly used for fungal cultivation (Saidu, Salim, & Yuzir, 2011). However, most of these substrates are not available in urban areas compared to other housing wastes such as wild grass or dried leaf. By finding new alternative substrate, we could lower the cost in mushroom production which will bring benefit and provide opportunity for urban area people to start growing mushroom for small scale business or self-sufficiency for food production. By further studying and development of cultivation technique, people could also can start to grow their own mushroom at home as the substrate could be easily found around and can be collected in large and sufficient amounts such as fern leaf, grass or dried leaf.

*Hypsizygus tessellatus* commonly known as Buna shimeji mushroom is a commonly cultivate mushroom in East Asian. It contain various kind of vitamins, polysaccharides, and amino acids which has high medicinal value such as antitumor, antiaging, controlling cholesterol, weight loss and constipation (Khondkar et al., 2012). Beside its medicinal value, *H. tessellatus* also been as a food source in the Eastern or Western countries. It said to have a crunchy texture and a sweet taste just like crab meat when it is cooked (Waites et al., 2001). Therefore *H. tessellatus* is a valuable mushroom species which has a market demand and potential to be widely cultivate by reducing its cultivation cost and increasing the harvesting yield.

If different strength of PDA gave the same effect for the mycelium growth, the half strength PDA can be used instead of the full strength PDA which will be more cost effective. Substrates such as *Dicranopteris linearis* (fern leaf), dry leaf of *Terminalia catappa* and *Imperata cylindrica* (lalang grass) have not been studied as an alternative substrate for the culturing of *H. tessellatus*. Therefore, the aim of this research was to determine the effect of different parts of the stalk and different strength of PDA on the mycelium growth of *H. tessellatus* and to determine the effect of *D. linearis*, dry leaf of *T. catappa* and *I. cylindrica* as an alternative substrate for the culturing of shimeji mushroom, *H. tessellatus*.

## Methodology

### *H. tessellatus (Buna Shimeji) and Wheat Grain*

The mushroom, *H. tessellatus* and the wheat grain used to produce the mushroom spawn were bought from Giant Supermarket in Nilai.

### *Mushroom Growth Substrate*

The substrate used for this study were dried leaf, lalang grass and fern leaf. Dry leaf of *T. catappa* were collected beside the sport hall of INTI University. *I. cylindrica*, lalang grass were collected from a grassy area at Melati area. *D. dichotoma*, fern leaves were collected at Melati hill. The Saw dust and rice bran which serve as control substrate were bought from the mushroom supplier.

### *Culturing H. tessellatus on different substrates*

This culturing method is modified from Dehariya, Chaubey, & Vyas (2010). The substrate collected were washed with water to remove dirt and dust. Each substrate were blended into powder or cut into small pieces before filling into capsules except for the sawdust and rice bran which were already obtained in powder form. 7 types of substrate were prepared: saw dust + rice brain (C), small pieces of *D. linearis* fern leaf (FA), *D. linearis* fern leaf in powder form (FB), small pieces of *I. cylindrica* grass (LA), *I. cylindrica* grass powder form (LB), small pieces of *T. catappa* dried leaf (DA) and *T. catappa* dried leaf powder form (DB).

Each kind of substrate were filled to 7 cm height from the bottom of the capsule. Capsule containing the substrate were autoclaved for 30 minutes under 121˚C at 10.342 kPa. Each autoclaved capsulate was filled with 25 spawn using antiseptic technique in the laminar air flow cabinet. The capsulate were tied using raffia string and kept in a tray covered with a black plastic sheet. The capsules were incubated at room temperature. Each treatment was replicated 11 times.

Capsules were sprayed with water once a day to maintain their moisture. The growth of mycelium in each capsule was observed. 11 samples using saw dust mixed with rice bran as substrate were used as control in this experiment. After 6 weeks of culturing, the final growth of the mycelium were recorded. A grid of 16 × 2 × 3.5 cm total 8cm × 7cm were created to measure the surface coverage of the mycelium growth. When mycelium covered more than half a grid, it will be counted as 1 grid. The horizontal growth of the mycelium was also measured in cm and recorded.

### *Statistical analysis*

Data collected was analyzed using SPSS. The two-way analysis of variance (ANOVA) was conducted to compare the significance of the differences of the mean of the mycelium growth on different strength of PDA and the different part of stalk mycelium. One- way ANOVA was conducted to compare the significance of differences of the mycelium growth on the upper surface and the length of horizontal growth of mycelium on different substrate. Turkey test was conducted to know which substrate gave no significant different mycelium growth compare the control substrate to be chosen as the alternative potential substrate for *H. tessellatus* cultivation.

## Results and Discussion

***Effect of different substrate on the growth of H. tessellatus in capsule***

7 types of substrate were test in this study, namely saw dust + rice brain (C), small pieces of *D. linearis* fern leaf (FA), *D. linearis* (fern leaf) in powder form (FB), small pieces of *I. cylindrica* (LA), *I. cylindrical* in powder form (LB), small pieces of *T. catappa* dried leaf (DA) and *T. catappa* dried leaf in powder form (DB).

Spawn in C and LB started to have mycelium activity on day 4 after the spawn were placed in the capsule followed by with FA on day 5, LA on day 6, DA and DB on day 7 and finally FB in day 10. By observing the pattern of the mycelium, substrate in small piece shown in figure 7, 9 and 11 have thicker mycelium formation compare to the powered form substrate shown in figure 6, 8, 10 and 12 including the control substrate C.

The highest contamination were observed in FB (5 samples) and DB (5 samples) after two weeks. Most of the contamination appeared to be black, green and white mold. At day 18, there were maggots growing in one of the LB contains, however, the growth of the mycelium was not much affected. This is because the maggots feed on the LB substrate but not on the growing mycelium.

Figure 1 shows a bar chart plotted from the mean percentage of mycelium coverage on the upper surface of different substrate, FA shows the highest percentage and FB gave the lowest percentage of mycelium coverage. FA, LA and DA are chosen to be the potential alternative substrate for *H. tessellatus*, as all of these substrate have more than 70% of mycelium coverage on the surface including the control substrate C. Figure 5 shows the bar chart plotted from the mean length of horizontal growth of mycelium in different substrate in capsule, C gave the longest length of mycelium growth but DB gave the least length of mycelium growth and FB shows no horizontal growth of mycelium at all. The result from Figure 2 was more or less same as Figure 1 where all substrate in small pieces (FA, LA and DA) has better mycelium growth effect than the powder form substrate (FB, LB and DB).

Statistical analysis of ANOVA showed that different substrates have significant effect on the mycelium growth on the upper surface and the length of horizontal growth of mycelium. The growth of the mycelium on different substrates are illustrated in figures 3 to 9 after five weeks.

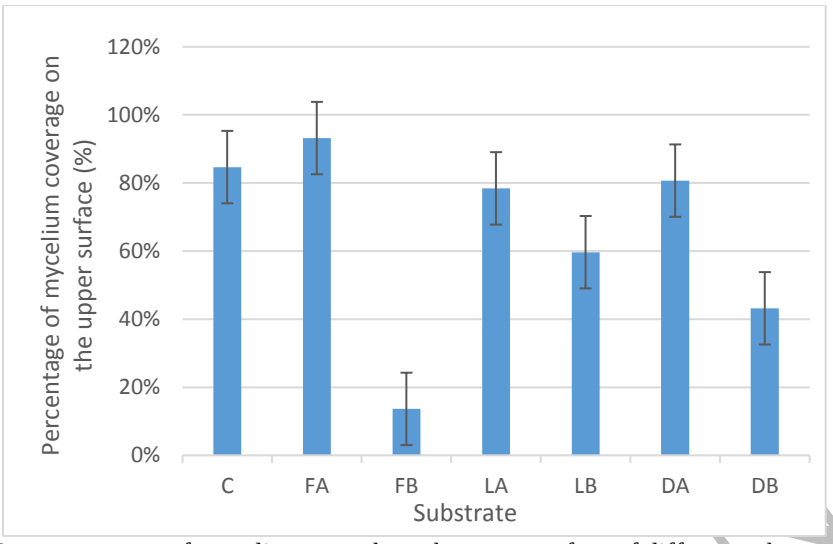


Figure 1. Mean percentage of mycelium growth on the upper surface of different substrate in capsule

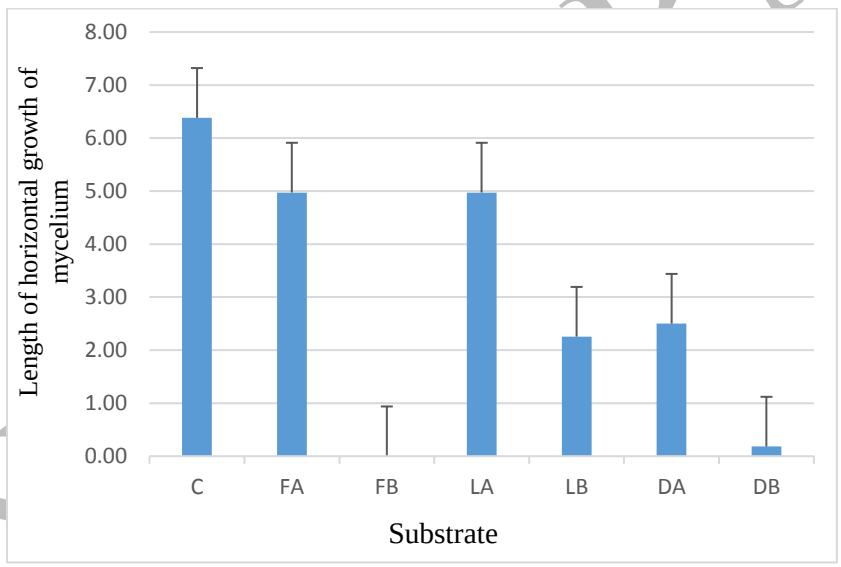


Figure 2 Mean length of horizontal growth of mycelium in different substrate in capsule


Figure 3 Mycelium growth in C after 5 week


Figure 4 Mycelium growth in FA after 5 week

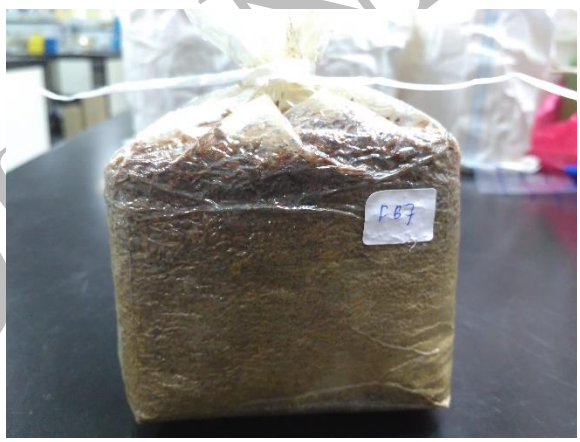


Figure 5 Mycelium growth in FB after 5 week


Figure 6 Mycelium growth in LA after 5 weeks


Figure 7 Mycelium growth in LB after 5 weeks

Figure 8 Mycelium growth in DA after 5 weeks

Figure 9 Mycelium growth in DB after 5 weeks

The results showed that FA (small piece of *D. linearis*) (figure 4) induced the best growth for the mycelium coverage at the upper surface of substrate in capsulate, although FA was found to produce the second highest in the horizontal growth of mycelium equivalent to that of LA (small pieces of *I. cylindrica*) (figue 6). The growth of mycelium were highly dependent on the total carbon, total nitrogen, C/N ratio and pH of the substrate (Oei, Nieuwenhuijzen, Feijter, & Zylva, 2005). According to Jambaro, Neri, & Alvarez (2014), the mycelium growth have a rapid degradation of lignin and slow degradation of cellulose and hemicellulose, substrate with high lignin composition have better growth effect compare to cellulose. *D. linearis* was reported to have high lignin composition (Russell, & Vitousek, 1997), and the nutrient composition in the fern are also richer than the nutrient in saw dust (Lin, 2004) which support the result for the better mycelium growth in FA than the control C. In this research, C exhibited the good mycelium growth compare to the rest of the substrate.

C contain mixture of saw dust and rice brain which is a god supplement for the mycelium growth. Studies has been reporting addition of rice bran in saw dust actually gave a better stimulation on the spawn running and the fruiting body formation (Imtiaj et. al, 2016). The fruiting body of *Oudemansiella radicata* were formed very well in the oak sawdust medium mixed with 10% rice bran (Semerdzieva *et al*. 1988), fruiting body of *Hericium erinaceus* was reported to have better growth when 10% of rice bran was added as supplement (Imtiaj et. al, 2008) and the production of shiitake mushroom was also increased by adding 30% of rice bran to the culture substrate (Fasidi and Kadiri, 1993). Therefore, rice bran probably contains some ingredient which could act as supplement for substrate to facilitate the growth of the mycelium (Rossi, Monteiro, Machado, Andrioli, & Barbosa, 2003).

LA also show good effect on the mycelium coverage and horizontal growth length. *I. cylindrical* were also reported to have high content of lignocellulosic materials which about 22% of the whole plant (Lin, & Lee, 2011) and this supported the better growth of mycelium. The *T. catappa* dried leaf shows the lowest effect to the mycelium growth. Narain *et al.* (2008) reported that the litter of dried leaf used as substrate for oyster mushroom cultivation gave the worst growth due to its high C:N ratio and cellulose, which seem to support the result obtained in this study.

The size of the substrate also gave the significant effect on the mycelium growth. The substrate, namely *D. linearis*, *I. cylindrical*, and dried leaf of *T. catappa* which were cut into

smaller pieces was found to have better growth rate of mycelium and thicker mycelium formation compared to the powdered form of the same substrate. According to Tesfaw, Tadesse, & Kiros (2015), the spawn of oyster mushroom dint grow well due to too much wheat bran was used causing the substrate to be compact. The study also state out that air circulation should be created to ensure the better growth of the mycelium. This is in agreement with our study where powder form are more compact which gave no air circulation than the small piece substrate. This also explain the reason why FB (powder form of *D. linearis*) produced the worst growth of mycelium as the powder form of fern is much finer than LB (powder form of *I. cylindrica*) and DB (powder form of *T. catappa*'s dried leaf). After the autoclave process, the capsule were compressed, air will be pressure out from the capsule, FB which in a fine powder form have no space for compression, therefore the FB powder substrate were compressed to a brick of fern powder substrate which have totally no air room within it.

The types showed significant difference in the growth of the mycelium, therefore a Turkey test was conducted to find the substrate which have the significant effect in mycelium growth compared with the commercial substrate, C. From the Turkey test, C, FA, LA, LB and DA has no significant effect of the mycelium growth based on the upper surface of the capsule; but in the length of the mycelium in horizontal growth C has only shows no significant different with FA and LA. Therefore FA and LA can be ensure to be have the same effect as the commercial substrate, C. Although in the experiment, fruiting body was not produced due to limitation of time, but the yield of the mushroom is directly related to the effect of the mycelium growth in the substrate (Thomas et al., 1998). Therefore, it is predicted that the yield of the *H. tessellatus* growing on FA and LA will be almost the same as growing in the commercial substrate C, mixture of sawdust with rice bran 10% of rice bran could be added to FA and LA in order to get better yield of mushroom as suggested by Rossi et al. (2003) in future study.



## Conclusions

This study is conducted for offering the alternative substrate for *H. tessellatus* cultivation. Substrate in small pieces has better effect in cultivation of *H. tessellatus* compared to the powder form substrate. Small pieces of *D. linearis* (fern leaf) and *I. cylindrica* (lalang grass) were found suitable to be used as alternative substrate for *H. tessellatus* cultivation where the mycelium growth in these substrate have no significant difference from the commercially used substrate which is mixture of saw dust and rice bran. Three of the alternative substrate mention above can be found in urban area in large amount which gave opportunity to people couldn't gain access to the commercial substrate chance to collect their own substrate for mushroom cultivation.

## Acknowledgements

The authors wish to acknowledge the financial support provided through the INTI IU Research Seeding Grant 2014, [Project no.: INTI Research Grant 2014(2):INT-FOSTEM-04-02-2014], by INTI International University, Malaysia.

## References


Dehariya, P., Chaubey, A., & Vyas, D. (2010). Pleurotus sajor-caju: An alternative source for bioconversion of wastes. *Mushroom Research - An International Journal, 19*, 90-93.

Fasidi, I., & Kadiri, M. (1993). Use of agricultural wastes for the cultivation of Lentinus subnudus (Polyporales: Polyporaceae) in Nigeria. *Rev. Biol. Trop., 41*, 411–415.

Imtiaj, A., Sultana, S., Hossain, J., Rahman, S., & Ohga, S. (2016). Performance of Vegetative Growth and Artificial Fruit Body Formation of Hypsizygus marmoreus in Bangladesh. *Journal Of Faculty Of Agriculture, Kyushu University, 61*(2), 257–262.

Jambaro, Neri, & Alvarez. (2014). Utilization of selected urban wastes as substrate solutions in the growth and yield performance of pleurotus sajor-caju (fr.) singer (gray oyster mushroom). *PUP J. Sci. Tech., 7*(1), 28- 44.

Khan, S. M., A. Nawaz, M.A. Ali, T. Ahmad, N.A. Khan & Atiq-ur-Rehman. (2012). Response of Oyster mushroom on different agricultural wastes of southern Punjab. Pak. *Journal of Agriculture Science, 49*, 127-130.

Khondkar, P., Monira, S., Haque, A., Muhit, A., Sarker, N., Alam, A., & Rahman, A. (2012). Antimicrobial, Antioxidant and Cytotoxic Properties of *Hypsizygus tessulatus* Cultivated in Bangladesh. *Research Journal Of Medicinal Plant, 6*(4), 300-308. http://dx.doi.org/10.3923/rjmp.2012.300.308

Lin, Z. (2004). *Mushroom Growers' Handbook* (1st ed., pp. 101- 107). MushWorld.

Lin, Y., & Lee, W. (2011). Bioethanol Production from Alkali-Pretreated Rice Straw by Simultaneous Saccharification and Fermentation. *Journal Of Biobased Materials And Bioenergy, 5*(3), 396-402. http://dx.doi.org/10.1166/jbmb.2011.1162

Mat Amin, M., Azahar Harun, & Abdul Wahab, M. (2014). Status and potential of mushroom industry in Malaysia. *Economic And Technology Management Review, 9b*, 103- 111.

Mujić, I., Zeković, Z., Lepojević, Ž., Vidović, S., & Živković, J. (2010). Antioxidant properties of selected edible mushroom species. *Journal Of Central European Agriculture, 11*(4), 387-392.

Narain R., R. K. Sahu, S. Kumar, S.K. Garg, C.S. Singh and R.S. Kanaujia. 2008. Influence of different nitrogen rich supplements during cultivation of Pleurotus florida on maize cobs substrate. *Environmentalist*. 29:1–7.

Nasim, G., Malik, S., Bajwa, R., Afzal, M., & Mian, S. (2001). Effect of Three Different Culture Media on Mycelial Growth of Oyster and Chinese Mushrooms. *Journal Of Biological Sciences, 1*(12), 1130-1133. http://dx.doi.org/10.3923/jbs.2001.1130.1133

Oei, P., Nieuwenhuijzen, B., Feijter, J., & Zylva, N. (2005). *Small-scale mushroom cultivation* (1st ed.). Wageningen: Agromisa.

Rajapakse, J., Rubasingha, P., & Dissanayake, N. (2007). The Potential of Using Cost-Effective Compost Mixtures for Oyster Mushroom (Pleurotus spp) Cultivation in Sri Lanka. *Tropical Agricultural Research And Extension*, *10*, 29-32. http://dx.doi.org/10.4038/tare.v10i0.1868

Rossi, I., Monteiro, A., Machado, J., Andrioli, J., & Barbosa, J. (2003). Shiitake (lentinula edodes) production on a sterilized bagasse substrate enriched with rice bran and sugarcane molasses. *Brazilian Journal Of Microbiology*, *34*(1). http://dx.doi.org/10.1590/s1517-83822003000100014

Russell, A., & Vitousek, P. (1997). Decomposition and potential nitrogen fixation in Dicranopteris linearis litter on Mauna Loa, Hawai'i. *Journal Of Tropical Ecology*, *13*(04), 579-594. http://dx.doi.org/10.1017/s0266467400010737

Saidu, M., Salim, M., & Yuzir, M. (2011). Cultivation of oyster mushroom (Pleurotus spp.) on palm oil mesocarp fibre. *African Journal of Biotechnology*, *10*(71). http://dx.doi.org/10.5897/ajb11.1942

Sanchez, C. (2004). Modern aspects of mushroom culture technology. *Applied Microbiology And Biotechnology*, *64*(6), 756-762. http://dx.doi.org/10.1007/s00253-004-1569-7

Semerdzieva, M., A. S. Buchalo, P. Hobsch, O. A. Zakordonec, S. P. Wasser and V. Musilek (1988). Comparative study of cultures of four species of the genus *Oudemansiella. Folia Microbiol*., *33*, 115–120.

Tesfaw, A., Tadesse, A., & Kiros, G. (2015). Optimization of oyster (*Pleurotus ostreatus*) mushroom cultivation using locally available substrates and materials in Debre Berhan, Ethiopia. *Journal Of Applied Biology And Biotechnology*, 015-020. http://dx.doi.org/10.7324/jabb.2015.3103

Tetiana A. Krupodorova & Victor Yu. Barshteyn (2015). Alternative substrates for higher mushrooms mycelia cultivation. *J. Biosci. Biotechnol.*, *4*(3), 39-347.

Thomas, G. V., Prabhu, S. R., Reeny, M. Z. and Bopaiah, B. M. (1998). Evaluation of lignocellulosic biomass from coconut palm as substrate for cultivation of Pleurotus sajor-caju (Fr.) Singer. *World Journal for Microbiology and Biotechnology*, *14*: 879–882

Waites, Morgan, N., Rockey, J., & Higton, G. (2001). *Industrial Microbiology: An Introduction* (1st ed., pp. 169–170). Oxford: John Wiley and Sons.

Zervakis, G., Philippoussis, A., Ioannidou, S., & Diamantopoulou, P. (2001). Mycelium growth kinetics and optimal temperature conditions for the cultivation of edible mushroom species on lignocellulosic substrates. *Folia Microbiol*, *46*(3), 231-234. http://dx.doi.org/10.1007/bf02818539