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Use of pomegranate peel mixed with wheat straw as the substrate to cultivation of two *Pleurotus* species

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Abstract: The effects of two different agricultural wastes (pomegranate peels mixture with wheat straw) on yield and quality of two *Pleurotus* species (*Pleurotus ostreatus* and *Pleurotus australis*) were determined in this study carried out between 2018 and 2019 years. Seven mixtures of substrates were prepared. The experiment was arranged in complete random design (CRD) and the data were analyzed by ANOVA with CRD- factorial using fisher (LSD) multiple comparison tests at $P \leq 0.05$. The fastest spawn run was obtained from T_7 mixture in *P. ostreatus* with 13.33 days. Pinhead formation was also early in T_7-*P. ostreatus* combination with 3.33 days. The minimum harvesting period was recorded in T_4 and T_5-*P. australis* combinations with 3.33 days. The highest total yield was obtained from T_6-*P. ostreatus* combination with 310.51 g and the lowest total yield was found in T_1-*P. australis* combination with 17.00 g. Also, the highest percentage of biological efficiency was observed in T_6-*P. ostreatus* combination with 95.54%, and the lowest percentage of biological efficiency was found in T_1-*P. australis* combinations with 5.23%. After assessment of all data, use of pomegranate peels with wheat straw (50+50%) can be recommended for *P. ostreatus.* This kind of studies can be beneficial for mushroom growers for finding the best substrate to cultivate *Pleurotus* species.

Key words: *Pleurotus ostreatus, Pleurotus australis*, pomegranate peels, wheat straw, yield, biological efficiency

İki *Pleurotus* Türünün Üretiminde Buğday Sapı ve Nar Kabuğu Karışımlarının Yetiştirme Ortamı Olarak Kullanımı

Öz: 2018 ve 2019 yılları arasında yapılan bu çalışmada, iki farklı tarımsal atığın (buğday samanı ile nar kabukları karışımı) iki *Pleurotus* türünün (*Pleurotus ostreatus ve Pleurotus australis*) verim ve kalitesine etkisi belirlenmiştir. Yedi substrat karışımı hazırlanmıştır. Deney tam rastgele tasarımda (CRD) düzenlenmiş ve veriler P≤0.05'te fisher (LSD) çoklu karşılaştırma testleri kullanılarak, CRD faktörü ile ANOVA ile analiz edilmiştir. En hızlı spawn gelişimi, *P. ostreatus*'taki T7 karışımından, 13.33 gün ile elde edilmiştir. Mantar taslağı (pin) oluşumu, ilk T7-*P. ostreatus* kombinasyonunda 3.33 gün olarak gerçekleşmiştir. Minimum hasat süresi, T4 ve T5-*P. australis* kombinasyonunda 3.33 gün olarak gerçekleşmiştir. En yüksek toplam verim, 310.51 g ile T6-*P. ostreatus* kombinasyonundan elde edilmiş ve en düşük toplam verim, 17.00 g ile T1-*P. australis* kombinasyonunda bulunmuştur. Ayrıca, en yüksek biyolojik verim yüzdesi, %95.54 ile T6-*P. ostreatus* kombinasyonunda gözlemlenmiş ve en düşük biyolojik verim yüzdesi, %5.23 ile T1-*P. australis* kombinasyonunda bulunmuştur. Tüm verilerin değerlendirilmesinden sonra, *P. ostreatus* için nar kabuğunun buğday samanı (50 +% 50) ile kullanılması tavsiye edilebilir. Bu tür çalışmalar, mantar yetiştiricilerinin *Pleurotus* türlerinin yetiştiriliciliği için en iyi substratı bulmakta faydalı olabilir.

Anahtar kelimeler: *Pleurotus ostreatus, Pleurotus australis*, nar kabukları, buğday samanı, verim, biyolojik etkinlik

Introduction

Pleurotus species are cultivated on non-composting substrate. Moreover, *Pleurotus* spp. is economically important among other mushroom genus in the world. It has high adaptation capacity to grow in various conditions. Also, oyster mushroom has been cultivated on various agricultural wastes (Stamets, 1993). Cultivation of *Pleurotus* species has many advantages such as requiring short period to grow, less pests and diseases damage, and having economic advantages (Chang and Miles, 2004; Yang et al., 2013).

Pleurotus spp. is a saprophytic fungus, which is decomposing dead wood into nature. Different species of *Pleurotus* are good in terms of responding to the various substrates (Sitaula et al., 2018). Its substrate should contain cellulose and lignocellulose. Oyster mushroom is a rich source of nutrients and is also a good resource of protein, minerals and vitamins (Bellettini et al., 2016). In addition, *Pleurotus* species has notable taste and flavor. There are several mushroom species recognized in the world and most of them are suitable to cultivate and eat as food (Nadir et al., 2016).

Ecological requirements of *Pleurotus* species are different according to the various stages of growing period. The optimal temperatures of spawn run and pinhead forming are between 20 to 30^{0}C and 10 to 20^{o}C, respectively. Relative humidity varies in different growing stages. Relative humidity of substrates should be 60% to 75%, however it should be 85 to 95% during the fruiting step (Stamets, 2000). *Pleurotus* species need lower light in the fruiting stage (Kang, 2004).

After harvesting pomegranates fruits, they are assessed as fresh, pomegranate juice and in different areas. The residues of pomegranates are discarded in Iraq. Some studies showed that pomegranate peels contains phenols (249.4 mg/g), moisture (8.1 g/100 g dry peel), protein (3.46 g/100 g dry peel), lipid (3.36 g/100 g dry peel), ash (6.07 g/100 g dry peel), fiber 17.63 (g/100 g dry peel) and carbohydrate (59.98 g/100 g dry peel) (Romelle et al., 2016). Moreover, pomegranate peels has cellulose (7.8%), hemicellulose (8.1%), and lignin (22.1%) (Pereira et al., 2016). It men's that they can be useful for mushroom cultivation.

Wheat straw has been used commercially for *Pleurotus* spp. production in Iraq. The pomegranate is a major fruit in Halabja province of Iraq. Although pomegranate has been produced in wide areas of Halabja province, there is a little knowledge about utilization of pomegranate fruit peels for *Pleurotus* cultivation. Therefore, the aim of this study was to investigate using of pomegranate fruit peels in *Pleurotus* spp. cultivation (*P. ostreatus* and *P. australis*).

Material and methods

This study was carried out at Horticulture and Landscape Design Department of Technical College of Applied Sciences (Sulaimani Polytechnic University, please add country) during years 2018-2019. Pomegranate fruit peels were used alone and in combination with different concentrations of wheat straw to cultivate of two species of *Pleurotus* sp. (*P. ostreatus* and *P. australis*) (Table 1). The spawn of the mushroom species were prepared in microbiology laboratory of Technical College of Applied Sciences. The oven dried pomegranate peels were used as the substrate. Water was added to the substrates for having 60-75% relative humidity. Substrates prepared was filled into autoclaveable plastic bags (15x30 cm), each bags contained (1 kg) of substrate. Then, the bags were sealed by cotton and labeled.

Table 1. Substrate mixtures used in this study for cultivation of two *Pleurotus* species

Treatment	Substrate	Substrate ratio (%)
T_1	PP:WS:WB: G	88:0:10:2
T_2		76:12:10:2
T_3		**66:22:10:2**
T_4		**44:44:10:2**
T_5		**22:66:10:2**
T_6		**12:76:10:2**
T_7		**0:88:10:2**

PP= Pomegranate peel, WB= Wheat bran, G= Gypsum, WS= Wheat straw

After that, the bags were autoclaved at 121^{0}C for 30 minutes in 1.5 atmospheres and were allowed to cool.

Next day, the bags were inoculated by 5% spawn and then they were transferred to cropping room for cultivation until harvesting mushrooms. The ecological requirements of the cropping room were showed in Table 2. Before the substrates were used in this experiment, it was tested to find moisture content (Table 3).

Table 2. Environmental condition for *Pleurotus* species (Stamets, 2000)

Parameter	Spawn run	Pin-head formation	Fruiting body
Temperature (^{0}C)	18-24	10-15	15-21
Relative humidity (%)	60-75	90-95	85-95
CO_2	200,000 ppm	500-1000 ppm	≤2000
Ventilation time	1	4-8	4-5
Light (Lux)	10,000	500-1000	500-1000

Table 3. Moisture content of materials used in this study for *Pleurotus* cultivation

Substrate	Moisture (%)
Pomegranate peels	12.2±0.1
Wheat straw	10.5±0.2
Wheat bran	9.5±0.01
Gypsum	2.01±0.02

The data were collected to determination of the spawn running time, pinhead formation time, harvested time, yield and biological efficiency (%). This experiment was designed with seven treatments for each species with three replications in complete random design (CRD). Also, the data were analyzed by ANOVA using (XLSTAT-pro and JMP7 version 7.5.2 window) program with CRD-factorial, which used fisher (LSD) multiple comparison tests at P<0.05, and the figure where drawing by (Graph Pad Prism 5).

Results and Discussion

Results of *P. ostreatus* and *P. australis* cultivation on different ratios of pomegranate peels mixed with wheat straw have showed significant effect on spawn run time (Figure 1). The longest period was reported from T_1: *P. australis* (27.33 days). However, the *P. ostreatus* gave nearly similar results from T_3 (26.66 days). The shortest period was obtained from T_7: *P. ostreatus* (13.66 days). The rate of spawn running may be affected by various factors, including carbon: nitrogen ratio (Zanetti and Ranal, 1997). Besides, the spawn quantity and nutrition content in substrate are known as effective in increasing the shortness spawn run (Bellettini et al., 2016). Different analyses have also indicated that the substrates used to cultivate *Pleurotus* mushroom were significantly influenced by pomegranate peels. Bellettini et al. (2016) reported that both longest and shortest period of spawn run gives similar results.

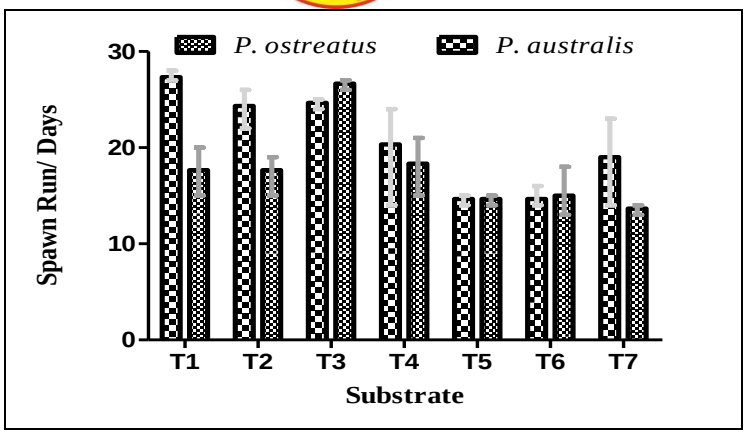

Figure 1. Effect of pomegranate peels mixed with wheat straw on spawn run of *P. ostreatus* and *P. australis*

The analysis result of the day required for the pinhead formation indicated that the highest percentage of pomegranate peels significantly impact on the *Pleurotus* species (Figure 2). The maximum pinhead formation was recorded in treatment T_1, T_2, T_3: *P. australis* and T_1: *P. ostreatus* (8.33 days). The minimum day required for the pinhead formation was obtained from Treatment T_6: *P. australis* (3.33 days). These results are similar with Sitaula et al. (2018), who use various substrates to cultivate of *Pleurotus* mushroom. However, Ahmed *et al.,* (2013) and Obodai et al. (2003) revealed that pinhead formation was observed in 7-10 days and 4-6 days in *Pleurotus* spp., respectively.

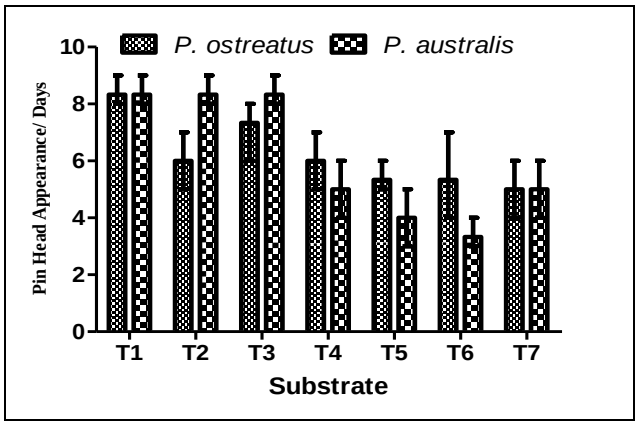

Figure 2. Effect of pomegranate peels mixed with wheat straw on pinhead formation in *P. ostreatus* and *P. australis*

Figure 3 showed period for the harvesting time from the end of pinhead formation. It can be noticed that T_4, T_5, and T6: *P. australis* showed the shortest harvesting time (3.33 days). The longest data was obtained from T_6: *P. ostreatus* (5.33 days). Findings of our experiment are similar with the results of Yang et al. (2013), who reported that the harvesting time of different strains of *Pleurotus* mushroom is influenced by temperature and relative humidity. Consequently, the moisture is an important factor for growth of *Pleurotus* mushroom and its production (Stamets, 2000).

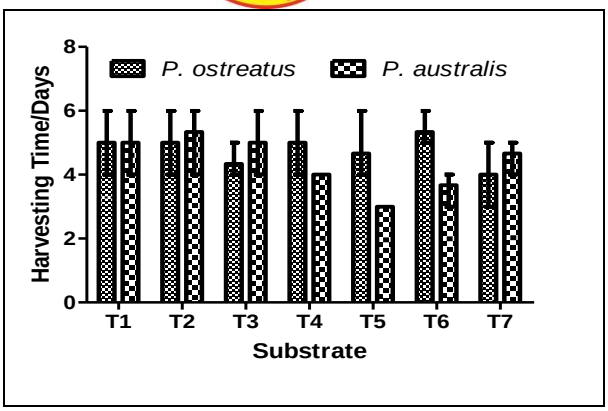

Figure 3. Effect of pomegranate peels mixed with wheat straw on harvesting time in *Pleurotus* cultivation (*P. ostreatus* and *P. australis)*

Yield mean values varied from 17.00 to 310.51 g among the substrates. Results of yield and biological efficiency illustrated in Figure 4 and Figure 5 were significantly affected ($P \leq 0.05$) by different combination of the pomegranate peels with wheat straw. In terms of total yield, the highest results were recorded in treatment of *P. ostreatus* T_6 (310.51 g) followed by T_7, T_2 and T_5 (308.46, 290.70 and 260.89 g, respectively). The lowest yield was obtained from T_1: *P. australis* (17.00 g). Carrasco et al. (2018) stated that the component of substrates strongly affected the yield and quality of harvested mushrooms. However, total yield decreased in this study when it is applied with an amount of pomegranate peels. Because, amount of phenolic compound helps to decay substrate (Li et al., 2006; Romelle et al., 2016). Yang et al. (2013) confirmed that the yield was affected by two main factors; first, high levels of nutrient available at higher rate; second, supplement of the substrates. Also, Dundar et al. (2008) recorded from 20.2 to 4.5 g total yield, which they used some supplements to cultivate three species of *Pleurotus* and their results were different from ours.

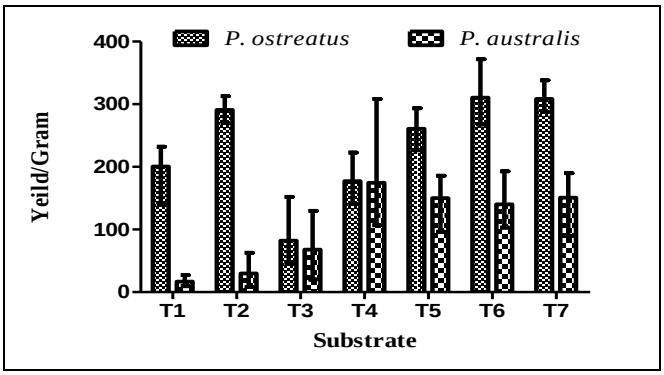

Figure 4. Yield values of *Pleurotus* mushroom species (*P. ostreatus* and *P. australis)* cultivated on pomegranate peels mixture with wheat straw

The percentage of biological efficiency in mushrooms consists of real factors to determination the yield and it is the ratio of the harvested mushrooms to the dry weight of growing substrate. In general, the highest yield is observed in the substrates that give the highest biological efficiency (Hoa et al., 2015). The percentage of biological efficiency ranged between 95.54 and 5.23%. The highest biological efficiency was obtained from wheat straw, which showed the significantly highest percentage of biological efficiency compared to other treatments. This

result agrees with my previous study for cultivation of *P. florieda* and *P. ostreatus.* In a stusy carried out by Baysal et al. (2003), the highest yield was recorded as 350.2 g, which used the substrate contained rice husk. Biological efficiency was found as 96.29-71.05% by Sitaula et al. (2018). Their results were similar to ours. Patar et al. (2018), who used the wheat straw to culture two species of *Pleurotus* and biological efficiency was ranged between 136.3 and 94.0%. Girmay et al. (2016) reported that the biological efficiency of oyster mushrooms cultivated on three different substrates ranged between 74.17 and 9.73%. Despite all these studies, there is no study on the cultivation of *Pleurotus* species using pomegranate peels.

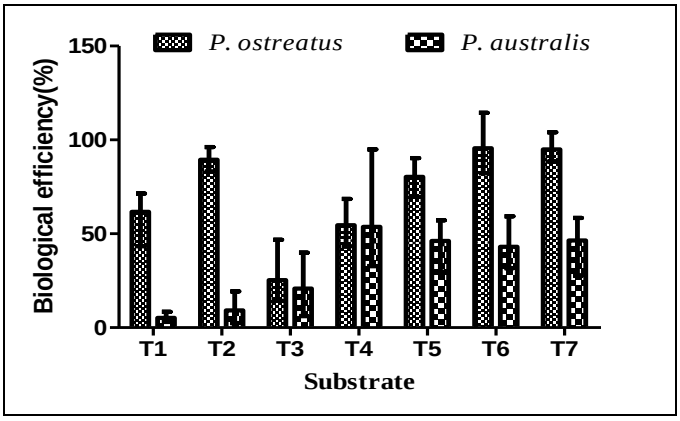

Figure 5. Biological efficiency (%) of *Pleurotus* species (*P. ostreatus* and *P. australis)* cultivated on pomegranate peels mixed with wheat straw

Conclusion

In fact, many agriculture wastes have high potential to be used as a substrate to mushroom cultivation. It is evidence that wheat straw is topmost as substrate for oyster mushroom. In this study, pomegranate peels were found as effective substrate material at 50% pomegranate peels + 50% wheat straw ratios for *Pleurotus* cultivation. It has been determined as effective on some parameters, especially yield. Therefore, pomegranate peels may be recommended as alternative locally available substrate for *Pleurotus* mushroom production.

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