

TOTAL PHENOLIC, FLAVONOID, TANNIN CONTENTS AND ANTIOXIDANT PROPERTIES OF *PLEUROTUS OSTREATUS* AND *PLEUROTUS CITRINOPILEATUS* CULTIVATED ON VARIOUS SAWDUST

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Abstract

In this study, the possibility of using of chestnut (*Castanea sativa*) sawdust in Oyster mushroom (*Pleurotus ostreatus* and *Pleurotus citrinopileatus*) cultivation was investigated. Additionally; *Pleurotus ostreatus* cultivation on the substrates which was mixed of chestnut with black poplar (*Populus nigra*) and oriental spruce (*Picea orientalis*) was performed. Bioactive properties of these mushroom and their growth mediums were also examined. After a successful harvest, total phenolic, flavonoid, condensed tannin contents and antioxidant properties of mushrooms' methanolic extracts were determined. Same analyses were also performed for mushrooms substrates. The highest yield and biological efficiency was observed in *P. ostreatus* cultivated on 100% *Castanea sativa* substrate. The highest total phenolic content (2.529±0.010 mg GAE/g) was found in *P. citrinopileatus* cultivated on *C. sativa* sawdust and its substrate medium. Total flavonoid could not determine any mushroom. The highest total condensed tannin (3.691±0.011 CE mg/g) content was observed in *P. ostreatus* cultivated on *C. sativa* sawdust and its substrate medium; the highest ferric reducing antioxidant power (11.761±0.020 µmol FeSO₄.7H₂O/g) was seen in *P. ostreatus* mushroom cultivated on 50% *P. orientalis* + 50% *C. sativa* and 100% *C. sativa* substrate medium. The highest free radical scavenging activity of DPPH was in *P. citrinopileatus* and 100% *C. sativa* sawdust and 100% *C. sativa* substrate medium.

Keywords: Antioxidant, chestnut, *Pleurotus*, tannin content, total phenolic content

ÇEŞİTLİ TALAŞLARDA ÜRETİLEN *PLEUROTUS OSTREATUS* VE *PLEUROTUS CITRINOPILEATUS* MANTARLARININ TOPLAM FENOLİK, FLAVONOİD VE TANEN İÇERİKLERİ VE ANTIOKSİDAN ÖZELLİKLERİ

Öz

Bu çalışmada *Pleurotus ostreatus* ve *Pleurotus citrinopileatus*'un (İstiridy mantarı/Kayın mantarı) kestane (*Castanea sativa*) odunu talaşındaki üretim olanakları üzerinde durulmuştur. Ayrıca; kestane talaşının; karakavak (*Populus nigra*) ve doğu ladini (*Picea orientalis*) talaşları ile karıştırıldığı ortamlarda *Pleurotus ostreatus* üretimi denenmiştir. Üretimlerin ardından elde edilen mantarların ve yetiştirme ortamlarının biyoaktif özellikleri araştırılmıştır. Başarılı bir hasat periyodundan sonra mantarların metanolik ekstraktları üzerinden toplam fenolik, flavonoid ve kondanse tanen içerikleri ve antioksidan özellikleri belirlenmiştir. Aynı deneyler mantar substratları için de tekrar edilmiştir. En yüksek verim ve biyolojik etkinlik değeri %100 *Castanea sativa* ortamında gelişen *P. ostreatus* mantarında gözlenmiştir. En yüksek fenolik içerik (2.529±0.010 mg GAE/g) *C. sativa* talaşında üretilen *P. citrinopileatus* mantarında ve kendi yetiştirme ortamında bulunmuştur. Hiç bir mantarda flavonoid içeriği tespit edilememiştir. En yüksek kondanse tanen içeriği (3.691±0.011 CE mg/g) %100 *C. sativa* talaşında üretilen *P. ostreatus*'da ve kendi yetiştirme substratında; en yüksek demir indirgeyici antioksidan aktivite (11.761±0.020 µmol FeSO₄.7H₂O/g) %50 *P. orientalis* + %50 *C. sativa* karışımında üretilen *P. ostreatus*'ta ve %100 *C. sativa* besin ortamında gözlenmiştir. En yüksek DPPH radikali temizleme aktivitesi %100 *C. sativa* ortamında üretilen *P. citrinopileatus* mantarında ve %100 *C. sativa* besin ortamında görülmüştür.

Anahtar kelimeler: Antioksidan, kestane, *Pleurotus*, tanen içeriği, toplam fenolik içerik

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INTRODUCTION

Under natural condition *Pleurotus* spp. is grown on living trees as parasite or dead woody branches of trees as primary decomposer and saprophyte (1). These species have extensive enzyme systems, so they are capable of utilizing lignocellulose-containing materials (2, 3). So, they can be cultivated on a wide variety of substrates containing lignin, cellulose and hemicellulose (4). In addition, cultivation of *Pleurotus* spp. can play an important role in managing lignocellulosic wastes (5).

Cultivation and consumption of mushrooms especially *Pleurotus* species' popularity have been increased day to day thanks to shorter cultivation time, medicinal and nutritional values and high yield potential of mushroom compared with *Agaricus* spp. (6-8). Mushrooms accumulate various secondary metabolites such as polyketides, phenolic compounds, steroids and terpenes (9). Many phenolic compounds have been reported to process potent antioxidant activity and to have anticancer or ant carcinogenic/anti-mutagenic, anti-atherosclerotic, anti-bacterial, anti-viral, and anti-inflammatory activities (10, 11). Some researchers have been noted that the bioactivity of phenolic may be related to their ability to inhibit lipoxygenase, chelate metals, and scavenge free radicals (12-14). Phenolic which present in mushrooms are known as natural antioxidants (15), so mushrooms have become more worth to researching. The composition of phenolic contents of mushrooms generally depends on genetic, environmental and other factors. The phenolic composition in mushrooms have been reported might be affected by a number of factors, namely mushroom strain/species, composition of growth media for in vitro cultured species, time of harvest, management techniques, handling conditions and preparation of the substrates for cultivated species and soil/substrate composition or host associated species in case of wild species either saprotrophic or mycorrhiza (16).

Most of natural compounds have a large number of biological activities involving antioxidant, anti-inflammatory, neuroprotective, chemopreventive and cardioprotective effects (17). Chestnut wood (*Castanea sativa*) can be considered one of them. *Castanea sativa* is a tree belonging to the *Fagaceae* family, living in generally Mediterranean

regions of Europe. It is a good source in terms of phenolic bioactive compounds, especially in tannins (17, 18). Its leaves have been widely used, in folk medicine, for some diseases such as bronchitis, asthma, cough, cold, expectorating (17, 19). Its leaves were described as a source of natural antioxidants (19). Additionally; an extract obtained from *Castanea sativa* bark has been shown antiviral effect against various viruses (20). In another study; it was recorded that sweet chestnut wood extract reduced oxidative stress and prevented DNA damage in blood lymphocytes (21).

Pleurotus spp. can grow on many different substrates. In this case; the quality, yield and mineral composition of mushroom varies according to the chemical structure and nutritional content of substrate (22-25). To our knowledge there is limited data in the literature about bioactive properties of *Pleurotus* spp. especially growing on *Castanea sativa* wood which is particularly rich in tannin. In this study it was investigated *Pleurotus* species (*Pleurotus ostreatus*, *Pleurotus citrinopileatus*) growing on *Castanea sativa* sawdust. Additionally; it was examined *Pleurotus ostreatus* growing on chestnut sawdust mixed with *Populus nigra* and *Picea orientalis* sawdust, separately. It was compared to the performance of *Pleurotus ostreatus* obtained from different substrates (100% *Castanea sativa* sawdust, 50% *Castanea sativa* sawdust + 50% *Populus nigra*, 50% *Castanea sativa* sawdust + 50% *Picea orientalis*) and performance of *Pleurotus citrinopileatus* obtained from 100% *Castanea sativa* sawdust in terms of yield, biological efficiency, total phenolic, flavonoids, condensed tannin contents and antioxidant properties. Same analyses were also performed for mushrooms substrates.

MATERIAL AND METHODS

Materials

P. ostreatus and *P. citrinopileatus* (Oyster mushroom) myceliums were obtained a commercial firm located in Istanbul. *Populus nigra*, *Picea orientalis* and *Castanea sativa* sawdust were supplied from plant of Forest Industry Engineering, Karadeniz Technical University, Trabzon.

Substrate preparation and mushroom cultivation

Particle sizes of wood sawdust approximately were 2-3 cm, homogeneously. *Populus nigra* (Black poplar), *Picea orientalis* (Oriental spruce) and *Castanea sativa* (Chestnut) sawdust moistened with water until 70-80% and sterilized in an autoclave at 121°C for 1.5 h, the pH of each medium then were adjusted to pH 6.5–7.0 by addition varying amount of CaCO₃ (26). After cooling the substrates to 20°C, they were placed in nylon bags of 1 kg and inoculated by spreading spawn on the surface of the substrate with a weight percentage of about 3% (w/w) of the wet weight of compost. Substrate condition was carried out in four replications. Each nylon bags were inoculated in mushroom growing laboratory (at 15-25°C, 70-80% relative humidity). Harvesting was started in fifth week and the fruit bodies' stipe and cap was calculated and weighed. Mushrooms and their cultivation mediums are presented in Table 1. Unfortunately; compost mediums, consisting of chestnut sawdust mixed with *Populus nigra* and *Picea orientalis* sawdust, prepared for *Pleurotus citrinopileatus* cultivation were contaminated, despite repeated two times.

Table 1. Mushrooms and cultivation mediums

Mushroom species	Cultivation medium (sawdust)
<i>P. citrinopileatus</i>	100% <i>Castanea sativa</i>
<i>P. ostreatus</i>	100% <i>Castanea sativa</i>
<i>P. ostreatus</i>	50% <i>Castanea sativa</i> + 50% <i>Populus nigra</i>
<i>P. ostreatus</i>	50% <i>Castanea sativa</i> + 50% <i>Picea orientalis</i>
<i>P. citrinopileatus</i>	50% <i>Castanea sativa</i> + 50% <i>Populus nigra</i> *
<i>P. citrinopileatus</i>	50% <i>Castanea sativa</i> + 50% <i>Picea orientalis</i> *

*: Contaminated variation

Total yield and biological efficiency

Mushroom yield was calculated as total fresh weight of mushrooms obtained from 3 or 4 flushes in the harvest period (26). Biological efficiencies were defined as the percentage ratio of the fresh weight of harvested mushrooms over the dry weight of substrates (27).

Preparation of the extract

Harvested mushrooms were sliced and dried in a food dryer 8 hours at 60 °C (Profilo, PFD1350W, Turkey). Dried mushroom was ground in a basic micro fine grinder and passed through 1

millimeter sieve (IKA, WERKE MF10, Germany). Approximately 5 g of powder samples in were placed into a falcon tube 50 mL 99% with additional methanol. The mixture was stirred continuously with a shaker (HeidolphPromax 2020, Schwabach, Germany) at room temperature for a total of 24 hours. Particles were removed using Whatman No. 4 filter paper pore size 20-25 µm. Then solutions were filtrated from hydrophilic polyvinylidene fluoride (PVDF) 0.45 µm for sterilization. The final volume of the solution was adjusted by the level of methanol.

Determination of polyphenolic contents

The polyphenolic contents of the methanolic samples were evaluated three different ways; total phenolic contents (TPC), total flavonoids (TF) and total condensed tannin (TT). For the determination of the total phenolic contents, the Folin-Ciocalteu procedure was employed and gallic acid was used as standard (28). Shortly, 20 µL of various concentrations of gallic acid and samples, 400 µL of 0.5 N Folin-Ciocalteu reagent and 680 µL of distilled water were mixed and vortexed. After 3 min incubation, 400 µL of Na₂CO₃ (10%) solution was added and vortexed. Then

the mixture was incubated for 2 h at 20 °C with interrupted shaking. Absorbance measurement was carried out at 760 nm at the end of the incubation period. A standard curve was prepared using gallic acid as a standard with different concentrations of gallic acid, and the results were expressed as mg (GAE) per g methanolic extracts.

The concentration of total flavonoid present in the methanolic extracts was measured using a spectrometric assay. Briefly, 0.5 mL samples, 0.1 mL of 10% Al(NO₃)₃ and 0.1 mL of 1 M NH₄CH₃COO were added to a test tube and incubated at room temperature for 40 min. Then the absorbance was measured against a blank at

415 nm. Quercetin was used for the standard calibration curve. The total flavonoid concentration was expressed as mg of quercetin equivalents per g sample (29)

Condensed tannins were determined according to the method by Julkunen-Titto(30). For each sample, various concentrations of 25 µL from extracts of plant were mixed with 750 µL of 4% vanillin (prepared with MeOH) and then 375 µL of concentrated HCl was added. The well-mixed solution was incubated at room temperature in darkness for 20 mins. The absorbance against the blank read at 500 nm. (+)-Catechin was used to help make the standard curve (0.05–1 mg/ml). The results were expressed as mg catechin equivalent to (CE)/g sample.

Determination of Antioxidant Capacity

The antioxidant capacity was determined using ferric reducing antioxidant power, free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH*).

Ferric reducing antioxidant assay (FRAP)

FRAP assay was also tested to determine the total antioxidant capacity of the samples. This method is based on the reduction of tripyridyltriazine complex (Fe (TPTZ)³⁺) to blue colored Fe(TPTZ)²⁺ by antioxidants in acidic medium (31). The preparation of working FRAP reagent was carried out by mixing 25 mL of 0.3 M acetate buffer pH 3.6 with 2.5 mL of 10 mM 2,4,6-tripyridylstriazine (TPTZ) solution in 40 mM HCl and 2.5 mL of 20 mM FeCl₃·6H₂O solution. The reaction mixture consisting of 1mL of the sample and 3 mL of freshly prepared FRAP reagent was incubated at 37 °C for 4 min. Then, the absorbance was determined at 593 nm against blank prepared with distilled water. A calibration

curve prepared with an aqueous solution of ferrous sulfate FeSO₄·7H₂O in the range of 100-1000 µM was used. Trolox was also tested under the same conditions as a standard antioxidant compound. FRAP values were expressed in wet weight of the samples as µmol of ferrous equivalent Fe (II) per g sample.

Scavenging of Free Radical (DPPH) Assay

The DPPH assay was applied using (32) to determine the radical scavenging capacity of the methanolic extracts of the mushroom. The simple method is based on scavenging the DPPH radicals with an antioxidant substance of the investigated solution. For each sample, six different concentrations of 0.75 mL of the extracts of the samples were mixed with 0.75 mL of 0.1 mM of DPPH in methanol, and the absorbance was read at 517 nm. The values were expressed as SC₅₀ (mg sample per mL), the concentration of the samples causing 50% scavenging DPPH radicals.

Statistical analysis

All assays were performed in triplicate. The data were recorded as means ± standard deviations and analyzed by using Statistical Package for Social Sciences (SPSS version 23.0). The yield data obtained were analyzed by ANOVA and tests of significance were carried out using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Total yield and biological efficiency

Total yield and biological efficiency of cultivated mushroom on sawdust are presented in Table 2.

Table 2. Total yield (g/100g substrates) and biological efficiency (%) of cultivated mushroom

Material	Yield (g/100g substrates) X̄±SD	B.E.* (%) X̄±SD
100% <i>C. sativa</i> sawdust**	18.4±1.2 ^a	65.0±4.2 ^{ab}
100% <i>C. sativa</i> sawdust***	20.6±2.3 ^b	72.8±8.1 ^b
50% <i>C. sativa</i> + 50% <i>P. nigra</i> sawdust***	19.7±1.8 ^{ab}	69.5±6.3 ^b
50 % <i>C. sativa</i> + 50% <i>P. orientalis</i> sawdust***	16.1±0.9 ^c	57.1±3.1 ^a

*: Biological efficiency

** : Values for *P. citrinopileatus* cultivation

***: Values for *P. ostreatus* cultivation

^a Means having the same superscript letter(s) are not significantly different ($P>0.05$) by Duncan's multiple range test.

After harvest period; the total yield (g/100g substrates) was calculated. 100% *Castanea sativa* sawdust used for *P. ostreatus* cultivation produced highest yield (20.6±2.3), whereas 50% *Castanea sativa* + 50% *Picea orientalis* sawdust produced the lowest (16.1±0.9) for the same mushroom. *Picea orientalis* additive had a negative effect on yield. This result can be attributed to the cultivation oyster mushroom on conifer wood substrates (pine, spruce, fir, etc). Such substrate types that have resins and other type of wood constituents can inhibit mycelium growth and colonization. Our results are comparable with other *P. ostreatus* cultivation studies (33, 34). Generally, total yield of different substrates was found significantly different ($P<0.05$) each other by Duncan's multiple range test. Biological efficiency (%) ranged from 57.1±3.1 to 72.8±8.1 and is similar with literature data (35). According to the Samuel and Eugene (2012); differences in biological efficiencies of the various substrates were due to different substrate compositions (36).

Polyphenolic contents

Total polyphenol (mg GAE/g), total flavonoid (mg QE/g) and condensed tannin (CE mg/g) contents of mushrooms and their own growth medium are presented in Table 3.

Phenolic compounds such as flavonoids, phenolic acids and tannins have been reported as natural antioxidants which commonly found in mushrooms with redox properties that act as reducing agents, hydrogen donors, free radical scavengers and singlet oxygen quenchers (15). In this study, *P. citrinopileatus* cultivated on 100% *C. sativa* sawdust exhibited the highest total phenolic content with 2.529±0.010 mg GAE/g and *P. ostreatus*

cultivated on 50% *C. sativa* + 50% *P. orientalis* sawdust was showed the lowest total phenolic content with 1.232±0.060 mg GAE/g. Our results are lower than some wild mushrooms' content (2.83-25.38mg GAE/g; (37, 38). Surprisingly; mushrooms have no detectable flavonoids, (Table 3). In the literature; total flavonoid content of *P. ostreatus* which grown on different substrates were ranged from 0.130±0.006 to 0.134±0.001 (mg QE/g) (39). In another study; total flavonoid content for *Pleurotus florida* was 0.17±0.02 (mg QE/g) and for *Flammulina velutipes* was 0.20±0.05 (mg QE/g) (40).

P. ostreatus and *P. citrinopileatus* cultivated on 100% *C. sativa* sawdust was produced the highest condensed tannin content (3.691±0.011 CE mg/g, 3.674±0.009, respectively). *P. ostreatus* cultivated on 50% *C. sativa* + 50% *P. nigra* sawdust was produced the lowest condensed tannin content with 0.618±0.062 CE mg/g. Our results were higher than cultivated *P. ostreatus* tannin content (0.32-1.44 CE mg/g) and higher than some wild mushrooms (*Lentinus ciliates*, *Hygrocybe conica*, *Schizophyllum commune*) tannin content (0.28-2.24 CE mg/g) (41), (Table 3). Generally, total phenolic content of mushrooms was found significantly different ($P<0.05$) each other by Duncan's multiple range test. This difference can be related to different content of growth medium.

When examined the mushroom growth medium; the highest total polyphenol and total tannin contents were obtained from 100% *C. sativa* sawdust substrate. 50% *C. sativa* + 50% *P. orientalis* sawdust substrate produced the lowest total polyphenol and total tannin contents. But the same substrate also exhibited the highest flavonoid

Table 3. Total polyphenol (mg GAE/g), total flavonoid (mg QE/g) and condensed tannin (CE mg/g) contents of mushrooms and their own growth medium

Mushroom / Growth medium	Total Polyphenol (mg GAE/g) $\bar{X}\pm SD$	Total Flavonoid (mg QE/g) $\bar{X}\pm SD$	Condensed Tannin (CE mg/g) $\bar{X}\pm SD$
<i>P. citrinopileatus</i> cultivated on 100% <i>C. sativa</i> sawdust	2.529±0.010 ^a	-	3.674±0.009 ^a
<i>P. ostreatus</i> cultivated on 100% <i>C. sativa</i> sawdust	1.768±0.082 ^b	-	3.691±0.011 ^b
<i>P. ostreatus</i> cultivated on 50% <i>C. sativa</i> + 50% <i>P. nigra</i> sawdust**	1.304±0.084 ^c	-	0.618±0.062 ^c
<i>P. ostreatus</i> cultivated on 50% <i>C. sativa</i> + 50% <i>P. orientalis</i> sawdust	1.232±0.060 ^c	-	1.380±0.009 ^c
100% <i>C. sativa</i> sawdust	25.153±0.041 ^a	0.643±0.097 ^a	3.758±0.021 ^a
50% <i>C. sativa</i> + 50% <i>P. nigra</i> sawdust	16.713±0.018 ^b	0.654±0.019 ^a	0.591±0.033 ^b
50% <i>C. sativa</i> + 50% <i>P. orientalis</i> sawdust	13.374±0.152 ^c	0.782±0.016 ^b	0.140±0.028 ^c

^aMeans having the same superscript letter(s) are not significantly different ($P>0.05$) by Duncan's multiple range test.

content. The small amount of flavonoid detected in the growing mediums. Also, the total phenolic content and condensed tannin of mushrooms' growing mediums were found significantly different ($P<0.05$) each other by Duncan's multiple range test.

The composition of phenolic contents of mushrooms generally depends on genetic, environmental and other factors. The phenolic composition in mushrooms might be affected by a number of factors, namely mushroom strain/species, composition of growth media for in vitro cultured species, time of harvest, management techniques, handling conditions, and preparation of the substrates for cultivated species (16).

Antioxidant properties

The antioxidant activity of cultivated mushrooms and their own growth mediums are presented in Table 4.

(Table 4). This results are similar with *H. erinaceum* mushroom chloroform extract's value (10.66 $\mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O/g}$, (43)) and lower than different sub fractions of methanol extracts of *Naematoloma sublateritium* (44.25-299.24 $\mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O/g}$, (44). Frap activities of mushroom and mushrooms' growth mediums extracts were found significantly different ($P<0.05$) by Duncan's multiple range test.

P. citrinopileatus cultivated on 100% *C. sativa* sawdust produced the highest DPPH value with 6.480 ± 0.089 mg/mL and *P. ostreatus* cultivated on mixed sawdust exhibited the lowest value with 22.922 ± 0.001 mg/mL, (Table 4). Our values were lower in terms of antioxidant activity than some wild mushrooms *Leucopaxillus giganteus*, *Sarcodon imbricatus*, *Agaricus arvensis* (1.44-3.50 mg/mL; (38) and similarly other than some cultivated mushroomssuch as *Agaricus bisporous* and *Agaricus brasiliensis* (1.67-4.57mg/mL, respectively; (45)). Generally, DPPH activities of

Table 4. The antioxidant activity of cultivated mushrooms and their own growth mediums

Material	FRAP ($\mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O/g}$) $\bar{X} \pm \text{SD}$	DPPH-SC ₅₀ (mg/mL) $\bar{X} \pm \text{SD}$
<i>P. citrinopileatus</i> cultivated on 100% <i>C. sativa</i> sawdust	10.130 ± 0.165^a	6.480 ± 0.089^a
<i>P. ostreatus</i> cultivated on 100% <i>C. sativa</i> sawdust	5.580 ± 0.189^b	19.167 ± 0.051^b
<i>P. ostreatus</i> cultivated on 50% <i>C. sativa</i> + 50% <i>P. nigra</i> sawdust	5.929 ± 0.051^c	22.922 ± 0.002^c
<i>P. ostreatus</i> cultivated on 50% <i>C. sativa</i> + 50% <i>P. orientalis</i> sawdust	11.761 ± 0.020^d	22.922 ± 0.001^c
100% <i>C. sativa</i> sawdust	733.200 ± 0.121^a	0.048 ± 0.001^a
50% <i>C. sativa</i> + 50% <i>P. nigra</i> sawdust	287.408 ± 0.043^b	0.109 ± 0.003^b
50% <i>C. sativa</i> + 50% <i>P. orientalis</i> sawdust	270.550 ± 0.098^c	0.144 ± 0.001^c

^a Means having the same superscript letter(s) are not significantly different ($P>0.05$) by Duncan's multiple range test.

The antioxidants are possible protective agents to helping the human bodies in decrease oxidative destruction (42). In this study, antioxidant capacity was determined using ferric reducing antioxidant power (FRAP), free radical scavenging activity of DPPH. FRAP refers to the antioxidant effect exerted by the donation of a hydrogen atom and subsequent breakage of the free radical chain (43).

The highest ferric reducing antioxidant power (11.761 ± 0.020 $\mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O/g}$) was determined in *P. ostreatus* cultivated on 50% *C. sativa* + 50% *P. orientalis* sawdust extract. The lowest one (5.580 ± 0.189 $\mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O/g}$) was seen in *P. ostreatus* cultivated on 100% *C. sativa* sawdust,

mushroom and mushrooms' growth mediums extracts were found significantly different ($P<0.05$) by Duncan's multiple range test, too.

CONCLUSION

In this study, the possibility of using of chestnut (*Castanea sativa*) sawdust in *Pleurotus ostreatus* and *Pleurotus citrinopileatus* cultivation was investigated. Additionally; *Pleurotus ostreatus* cultivation on the substrates which was mixed of chestnut with *Populus nigra* and *Picea orientalis* was performed. Bioactive properties of these mushroom and their growth mediums were also examined.

100% *Castanea sativa* sawdust which used for *P. ostreatus* cultivation produced highest yield (20.6 ± 2.3 g/100g) and biological efficiency (72.8%) whereas 50% *Castanea sativa* + 50% *Picea orientalis* variation produced the lowest (16.1 ± 0.9 g/100g) for the same mushroom. *Picea orientalis* additive had a negative effect on yield probably due to the resins and other phenolic components present in conifer wood substrate which can inhibit mycelium growth. *P. citrinopileatus* cultivated on 100% *C. sativa* sawdust exhibited the highest total phenolic content with 2.529 ± 0.010 mg GAE/g, in parallel to the growth medium. Total flavonoid could not determine any mushroom species. *P. ostreatus* and *P. citrinopileatus* cultivated on 100% *C. sativa* sawdust was produced the highest condensed tannin content correspondingly the growth medium. Since the tannin is widely found in chestnut wood, this result is not surprising. In the same substrate medium (100% *C. sativa* sawdust) the antioxidant activity of *P. citrinopileatus* was found two times higher than that of *P. ostreatus*. The highest ferric reducing antioxidant capacities (FRAP) 11.761 ± 0.020 $\mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O/g}$ was determined in *P. ostreatus* cultivated on 50% *C. sativa* + 50% *P. orientalis* sawdust at the same time *P. citrinopileatus* cultivated on 100% *C. sativa* sawdust FRAP values was found to be 10.130 ± 0.165 . *P. citrinopileatus* cultivated on 100 % *C. sativa* sawdust produced the highest DPPH value with 6.480 ± 0.089 mg/ mL. Consequently; *C. sativa* and the other sawdust types were generally showed good results in terms of studied various bioactive analyses. Different *Castanea* wood species can be performed with more efficient extraction methodologies and different mushroom species.

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