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The recycling potential of various local lignocellulosic residues for the cultivation of *Pleurotus nebrodensis* (Inzenga) Quél.

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Abstract

Various lignocellulosic waste products, generated from agricultural harvesting, present significant environmental challenges in terms of their use and disposal. The cultivation of mushrooms offers a cost−effective biotechnological method to recycle these lignocellulosic residues. This study aimed to assess the suitability of local lignocellulosic residues for the cultivation of *P. nebrodensis*, a relatively new species in mushroom cultivation. The study measured several parameters: spawn colonization periods (15.7−17.0 days), initiation of the first primordia (38.7−41.3 days), total harvest periods (77.3−83.3 days), and total yields (6.7−10.5 g/100 g) for *P. nebrodensis* grown on bean pods (BP), a mixture of bean pods and chickpea pods (BP−CP, 1:1), and a mixture of bean pods and wheat straw (BP−WS, 1:1). While there were no statistically significant differences in the first primordia initiation period, first harvest period, total harvest period, or second yield, significant differences were observed in spawn colonization period, first yield, second primordia initiation period, and total yields. These findings suggest that exploring less costly and more readily available alternative substrates, such as BP, CP, and WS wastes, could be advantageous for *P. nebrodensis* production. In conclusion, there is a need for further research on the cultivation of *P. nebrodensis* using various agricultural wastes, given its emerging status among cultivated mushrooms.

Keywords – agro−wastes – bioconversion of lignocellulosic wastes – edible mushroom – mushroom cultivation – *P. nebrodensis*

Introduction

Different agricultural products constitute a significant portion of the global agricultural economy. Many cereals obtained from agricultural harvests play a crucial role in human nutrition. The harvesting of these crops generates a large amount of lignocellulosic residues (Munir et al. 2024). Most of these agricultural waste products are either left where they are or incinerated, while a very small portion is utilised as animal feed (Mohd Hanafi et al. 2018, Kumla et al. 2020, Devi et al. 2023, Sarkar et al. 2024). The disposal and incineration of these waste residues not only causes serious environmental problems (air pollution, reduction and pollution of soil fertility (Khan et al. 2024, Jayaraman et al. 2024), destruction of organisms (micro−macro) that are beneficial for soil)

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but also contributes to human health risks, global climate problems and erosion (Ritoa & Manzi 2019, Blasi et al. 2023, Majib et al. 2023).

The utilisation of agricultural waste in mushroom cultivation represents a significant opportunity to address the environmental challenges associated with the disposal of bio−waste. In recent years, this approach has emerged as a promising strategy for reducing the environmental impact of agricultural activities. It is of great importance that agricultural wastes can be transformed into food sources by fungi without harming the environment (Kumla et al. 2020, Suwannarach et al. 2022) and that these wastes can be used in different sectors (Martin et al. 2023, Törős et al. 2024) (such as soil additives and fertilizers in agriculture, feed additives in animal husbandry and peat additives in floriculture) after bioconversion (Umor et al. 2021, Huang et al. 2023). These products are also indispensable for different value−added products (biofuels, biocomposites and bioplastics etc.) (Grimm & Wösten 2018, Yaashikaa et al. 2022, Blasi et al. 2023, Mujtaba et al. 2023). In recent years, mushroom cultivation has become an important branch of the agricultural economy, offering high economic returns, easy employment and no waste, as the products left over after production can be used in other sectors. In the cultivation of *Pleurotus* (Fr.) P. Kumm. species, agricultural waste products are not subjected to any pre−treatment, no chemical treatment, simple cultivation conditions, potential for production in 4 season, different organoleptic properties, different varieties with flavour and aroma content, high nutritional and medicinal values make them attractive (Mahari et al. 2020, Melanouri et al. 2022).

It is known that *Agaricus bisporus* (J.E. Lange) Imbach, *Pleurotus* (Fr.) P. Kumm. species and *Lentinula edodes* (Berk.) Pegler, are the three mushrooms with the highest production and market share in the world (Kumla et al. 2020, Mleczek et al. 2020, Sharma et al. 2023). The fact that the first requires pre−treatment in composting and the use of covering soil, the third has production potential only in certain countries, is not recognised in others, and has a limited market share, increases the importance of the production and consumption potential of *Pleurotus* spp.. These mushrooms include several species, such as *Pleurotus ostreatus* (Jacq.) P. Kumm., *P. pulmonarius* (Fr.) Quél., *P. djamor* (Rumph. ex Fr.) Boedijn, *P. citrinopileatus* Singer, and *P. eryngii* (DC.) Quél., etc. They do not require pre−treatment during composting, have strong enzyme activities, can be easily produced in different combinations, especially on lignocellulosic wastes, do not require chemical applications at any production stage, have short cultivation periods with early product yield, and can be produced by different methods. These advantages increases the importance of these species (Ritota & Manzi 2019, Mahari et al. 2020, Raman et al. 2021), and we believe they will capture a significant global market share in the next 20 years.

Some *Pleurotus* species (*P. ostreatus, P. pulmonarius*, *P. djamor*, *P. citrinopleatus*, and *P. eryngii*) are produced on a global scale and account for a significant market share (Correa et al. 2016, Ritota & Manzi 2019, Raman et al. 2021). They possess nutritional and medicinal properties and can be grown under simple conditions that are well accepted by consumers (Atila 2017, Ritota & Manzi 2019, Ragunathan & Swaminathan 2003, Magdziak et al. 2021, Koutrotsios et al. 2022, İnci et al. 2023, 2025). However, there is a need to introduce new species that are not currently produced to consumers. In recent years, the cultivated mushroom sector has been oriented towards the production of new species that can be grown in limited quantities or not at all, cultivated, and therefore lack market share. The trend in cultivated mushrooms is to produce different mushroom species (Gargano et al. 2013, Venturella et al. 2016, Zou et al. 2020, Cirlincione et al. 2022), including *P. eryngii* and its varieties (Guardia et al. 2005, Sardar et al. 2017, Koutrotsios et al. 2022, Atila & Çetin 2024, Akyüz & Kırbağ 2024, Jaffali et al. 2024), *P. nebrodensis* (Gargano et al. 2013, Koutrotsios et al. 2018, Jeoung et al. 2018, Sakellari et al. 2019, Kim et al. 2023), and *P. tuoliensis* (C.J. Mou) M.R. Zhao & J.X. Zhang (Wang et al. 2018, Zou et al. 2020), which are relatively new, and are being present to consumers in accordance with their preferences.

In recent years, the significance of oyster mushroom − *Pleurotus* species in the diversity of cultivated mushrooms has been steadily increasing worldwide. Their easy cultivation conditions, various strains, lack of need for pre−treatment, as well as their flavor, aroma, organoleptic

properties, and nutritional and medicinal benefits have contributed to their growing importance and cultivation (Zervakis & Venturella 2002, Correa et al. 2016, Zervakis & Koutrotsios 2017, Wang et al. 2018, Bellettini et al. 2019, Thakur 2020). Consumer demands, combined with rapid advancements and shifts in the gourmet and gastronomy sectors, are driving the market introduction of diverse mushroom species. The cultivation and promotion of genetically unaltered natural mushroom species, along with new varieties and flavors, are becoming increasingly important. *Pleurotus nebrodensis* is characterized by notably slow mycelial growth and heightened sensitivity to cultivation parameters−such as high light, relatively low temperatures, high humidity, and low CO_2−compared to other cultivated mushrooms. Its extreme rarity in nature enhances the value of *P. nebrodensis* (Gargano et al. 2013). Despite this, it does not hold a significant market share as a cultivated mushroom in many countries, including Türkiye. Thus, it is crucial to introduce new and lesser−known mushroom species to consumers. This study aimed to investigate the potential of utilizing various local lignocellulosic residues for the cultivation of *P. nebrodensis*.

Materials & Methods

Source of mushrooms and cultivation techniques

The parent culture of the *P. nebrodensis* (Inzenga) Quél. strain (WC980) was obtained from the Department of Plant Pathology and Environmental Microbiology at Penn State University (PSU), USA. This isolate kindly provided by Dr. Selime Semra Erol from Düzce University, Düzce, Türkiye, and was stored in malt extract agar (MEA) medium at 4 °C for future use. The strain was identified using molecular data (ITS1−5.8S−ITS2 and IGS1 rRNA sequences) and the DNA sequence information obtained was subjected to a blastn analysis via GenBank. This analysis demonstrated 100% similarity to the actual reference sequences (GenBank: KF743821 and HM998799) (Zervakis et al. 2014).

All experimental work related to the culture study was carried out in the Microbiology Application Laboratory and Fungal Culture Laboratory of the Science Technology Application and Research Centre at Bitlis Eren University, Bitlis, Türkiye. The remaining stages of the cultivation process, including pure mycelium growth, spawn propagation, preparation of compost and spawn inoculation procedures, culture conditions and harvesting, were carried out in accordance with the proposed methods summarised in reference (Zadrazil 1978, Delmas & Mamoun 1983, Philippoussis et al. 2001, Gargano et al. 2013, Koutrotsios et al. 2018, Sakellari et al. 2019), with some minor modifications.

Inoculum preparation

A 2.0% malt extract agar (MEA) solution was utilized for subculturing the pure culture. Approximately 0.5 cm² of mycelium−inoculated agar was excised from the main culture and transferred to the center of petri dishes containing 25 mL of MEA agar. The samples were then incubated at 25 °C for 25 days in the dark (Fig. 1a).

Spawn propagation

Initially, the wheat grains (WG) were thoroughly washed, cooked for 40 minutes, and air−dried, after which 2 grams of lime and 8 grams of gypsum per kilogram of dry grain were added to achieve the desired pH of 5.5–6.5, and the mixture was thoroughly mixed. The prepared grains (120 g) were transferred to 250 ml flasks and sterilized in an autoclave at 121 °C for 15 minutes. After cooling, two to three agar discs (1 cm²) from *P. nebrodensis* culture were aseptically transferred to the grains (Fig. 1b) and then incubated at 25 °C in the dark for 15 days to allow complete mycelial growth (Fig. 1c). On the fifth day (Fig. 1b), the flasks were shaken to maximize the colonization of the grains with the mycelium.

Substrate (compost) preparation and condition of cultivation

Three types of lignocellulosic substrates were utilized for cultivating *P. nebrodensis*: dry bean pods (*Phaseolus vulgaris* L.), chickpea pods (*Cicer arietinum*), and wheat straw (*Triticum aestivum* L.). These dried agricultural wastes were sourced from local farmers in Bitlis, Türkiye, during the harvest season from August to September. The primary reasons for choosing these materials are their abundance and cost−effectiveness. The substrates were categorized into two treatments: a mixture of bean pods and chickpea pods (BP−CP, 1:1), a mixture of bean pods and wheat straw (BP−WS, 1:1), and a control: bean pods only, as detailed in Table 1. To prepare the substrates, they were soaked in tap water for three days, after which excess water was removed. Lime and gypsum were added at a rate of 35 grams per kilogram of dry compost to achieve the desired pH range of 5.5−6.5, and the mixture was hand−mixed for 15 minutes. Each substrate group was then placed in separate cotton cloth bags, sterilized in an autoclave at 121 °C for 30 minutes, and allowed to cool to room temperature before inoculation. The cooled substrate was transferred onto a polythene cover, previously wiped with 95% ethyl alcohol, within a sterile inoculation chamber (Fig. 1d). For each experimental group, 1 kg of sterile compost was inoculated with 5% spawn and placed in 20 × 30 cm polythene bags. The compost was incubated in a culture room (2.35 × 2.42 × 3.17 m) at 25±1 °C in the dark for 15 days until it was fully colonized by the spawn (Fig. 1e). All experiments were conducted in triplicate. Upon complete colonization of the compost (Fig. 1e), the bags were opened and maintained at 14±1 °C with 80−85% humidity, 1000 lux light intensity (12 hours per day), and 3 hours of ventilation per day for primordium stimulation (Fig. 1f−g).

Fig. 1 − Cultivation process of *P. nebrodensis*. a mycelium growth. b−c spawn preparation and inoculation. d compost preparation. e spawn colonization, bag opening, and irrigation of the culture. f−g primordia formation.

Subsequently, the cultures were watered two to three times daily by spraying. Fruit bodies were harvested upon reaching their characteristic maturity stage (Fig. 2h–k). The average duration from inoculation to harvest was approximately 85 days. The cultivation process of *P. nebrodensis* was comprehensively described and effectively visualized in Fig. 1 and 2.

Fig. 2 − Fructification of *P. nebrodensis* in culture conditions. h−k development and maturation of basidiocarps.

Statistical evaluation

The data were analyzed using SPSS 25 statistical software, and results are presented as mean ± standard deviation. The significance of differences between groups was assessed using the Duncan post–hoc test, with a significance level set at $p < 0.05$.

Results & Discussion

Table 1 presents data on the spawn colonisation period, primordia initiation period, total harvest period (in days) and total yield (in g/100 g) of *P. nebrodensis* cultivated on dry bean pods (BP), a mixture of bean pods and chickpea pods (BP−CP, 1:1), and a mixture of bean pods and wheat straw (BP−WS, 1:1).

Table 1. Evaluation of local lignocellulosic substrates for the cultivation of *P. nebrodensis*.

Substrates	Spawn colonization period	First primordia initiation period	First harvest period	First Yield (g/100 g)	Second primordia initiation period	Total harvest periods	Second yield (g/100 g)	Total yield (g/100 g)
BP	17.0±0.0^{b}	38.7±1.5^{a}	50.0±1.7^{a}	6.3±0.8^{b}	70.7±1.2^{b}	83.3±3.1^{a}	4.2±1.6^{a}	10.5±0.9^{b}
BP−CP	16.6±0.6^{b}	41.3±6.0^{a}	54.0±5.6^{a}	6.4±1.8^{b}	63.0±4.6^{a}	77.3±4.7^{a}	4.2±0.8^{a}	10.5±2.3^{b}
BP−WS	15.7±0.6^{a}	40.0±1.7^{a}	54.7±2.5^{a}	3.7±0.3^{a}	67.7±1.2^{ab}	81.3±1.5^{a}	3.0±0.9^{a}	6.7±1.0^{a}
F value	6.500	0.384	1.421	5.511	5.676	2.471	1.043	6.061
p value	**0.031**	0.697	0.312	**0.044**	**0.041**	0.165	0.409	**0.036**

BP: bean pods (control group), CP: chickpea pods, WS: wheat straw, BP−CP (1:1): a mixture of bean pods and chickpea pods, BP−WS (1:1): a mixture of bean pods and wheat straw

ANOVA test statistic: reported as "F value (*p* value, n=3)"

Data Presentation: values are expressed as the mean ± standard deviation (SD) from three replicates ($n = 3$, $p < 0.05$)

Statistical significance: noted as [a] and [b] for significant differences between compost mediums ($p < 0.05$).

Total yield represents the cumulative yield obtained from both harvesting phases (first and second yield), and it was calculated in grams per 100 grams of substrate with 70% moisture content.

The mycelial growth period and spawn colonization period of *P. nebrodensis* were observed on MEA and wheat grain at 25 °C in the dark, with average durations of 25 days (Fig. 1a) and 15 days (Fig. 1c), respectively. In contrast, similar studies have reported that *P. nebrodensis* mycelia and spawn developed in 7−10 days on PDA and seed medium (Choi et al. 2006). These differences underscore the impact of the specific isolate and the nutrient medium used on growth rates.

The spawn colonisation period of *P. nebrodensis* grown on different lignocellulosic residues (BP, BP−CP (1:1) and BP−WS (1:1)) ranged from 15.7 to 17.0 days ($p < 0.05$, Table 1, Fig. 1e). The shortest colonization period was observed with BP−WS (1:1), at 15.7 days ($p < 0.05$, Table 1). The fruiting bodies of *P. nebrodensis* were typically harvested approximately 12 to 15 days after the first appearance of primordia (Fig. 1f). In comparable studies, the colonization of compost media by spawn was reported to take 26−29 days (Kim et al. 2023), 14−30 days (Jeoung et al. 2018), and 28−32 days (Koutrotsios et al. 2018), with primordia reaching harvest size in 12−18 days. Compared to these studies, the spawn colonized the compost media at an earlier stage in this study. No significant differences were observed in the time required for primordia to reach harvest maturity.

The first primordia initiation period for *P. nebrodensis* ranged from 38.7 to 41.3 days across various composts ($p > 0.05$), while the second primordia formation period ranged from 63.0 to 70.7 days ($p < 0.05$, Table 1, Fig. 1f−g). The earliest initiation of the first primordium was observed at 38.7 days on BP, whereas the earliest formation of the second primordium occurred at 63.0 days on BP−CP (1:1). The initial primordium formation period did not show a statistically significant difference among the different compost substrates ($p > 0.05$). However, there was a statistically significant difference in the second primordium formation period ($p < 0.05$), as detailed in Table 1.

The cultivation of *P. nebrodensis* lasted approximately 77.3 to 83.3 days, yielding an average of two harvests. The fruiting bodies (Fig. 2h−k) were typically harvested about 12 to 15 days after the initial appearance of primordia (Fig. 1f). The initial and total harvest periods for *P. nebrodensis*

grown on various lignocellulosic substrates were analyzed over a culture period of approximately 85 days, with no statistically significant differences observed (Table 1). In comparable studies (Jeoung et al. 2018, Koutrotsios et al. 2018, Kim et al. 2023), the culture period for *P. nebrodensis* ranged from 75 to 130 days, reflecting variations based on the mushroom isolate, culture medium, and growing conditions (Table 1).

The cultivation of *P. nebrodensis* was carried out for approximately 85 days, during which an average of two harvests were obtained. The total yield per 100 g of material (70% moisture) varied, with the lowest yield of 6.7 g observed on the BP−WS (1:1) substrate, and the highest yield of 10.5 g on BP and BP−CP (1:1) (Table 1). Statistically significant differences were found in the total yield among different local agricultural wastes ($p < 0.05$, Table 1). The results indicated that BP and BP−CP (1:1) substrates were the most effective, yielding a total of 10.5 g per 100 g of material (70% moisture). Various studies (Jeoung et al. 2018) have reported that the yield of *P. nebrodensis* from different culture media typically ranges from 7.0 to 13.5 g/100 g. Compared to the study by Jeoung et al. (2018), which explored different lignocellulosic waste combinations, no significant incompatibilities in total yield were noted in the current study. However, the total yield observed here (6.7−10.5 g/100 g) was lower than the 20.7-63.1 g reported by Koutrotsios et al. (2018) and Kim et al. (2023).

Conclusions

Consequently, these findings demonstrate that *P. nebrodensis* can be effectively cultivated using local agricultural waste (Fig. 1). This approach not only facilitates the recycling of lignocellulosic residues but also supports the production of protein−rich foods. *P. nebrodensis*, a relatively new species among cultivated mushrooms, displays distinctive characteristics that set it apart from other cultivated varieties. Its fruiting bodies (Fig. 2) are ivory−white with a firm, hard, and plump structure. The mushroom is noted for its attractive aroma and superior taste compared to other cultivated mushrooms, despite its lower yield (Table 1). To enhance yields in the relatively new cultivation of *P. nebrodensis*, pure or 1:1 mixtures of concentrated components, such as bean and chickpea pod wastes, can be utilized. Further research into *P. nebrodensis*−which features unique color, aroma, taste, and texture−will contribute to increased diversity among cultivated mushrooms.

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Author Contribution

MA and SK contributed to the conceptualization, supervision, methodology, analysis, writing, revision, and editing of the final manuscript. All authors reviewed and approved the final version of the manuscript prior to its submission.

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Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest

The corresponding author, on behalf of all authors, declares that there are no conflicts of interest.

Ethics approval

This manuscript does not involve experiments with human or animal participants.

Consent to participate

Not applicable

Consent to publish

All authors have provided their consent for the publication of this manuscript in the journal

Research involving human participants and/or animals

Not applicable

Informed consent

Not applicable

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