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Yerba mate residue (*Ilex paraguariensis* – Aquifoliaceae) in the production of edible mushroom *Pleurotus ostreatoroseus*

Resíduo de erva-mate (*Ilex paraguariensis* – Aquifoliaceae) na produção do cogumelo comestível *Pleurotus ostreatoroseus*

Residuos de yerba mate (*Ilex paraguariensis* – Aquifoliaceae) para la producción del hongo comestible *Pleurotus ostreatoroseus*

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Abstract
Mushrooms are cultivable on various plant substrates and the yerba mate residue may be one of the most promising, especially if domestic cultivation techniques are developed. As a large producer and consumer of yerba mate, southern Brazil also presents a large production of this residue. In this sense, this work aimed to test the cultivation of *Pleurotus ostreatoroseus* on yerba mate residue, seeking a technique for its domestic cultivation. Three substrates were prepared: a) partially decomposed yerba mate; b) recently discarded yerba mate; c) yerba mate recently discarded and added with eucalyptus sawdust. Cultivation of *P. ostreatoroseus* in yerba mate just after disposal and partially decomposed proved to be unviable, with good mycelial colonization of the substrate, but without basidioma production. In cultivation with sawdust, all concentrations tested showed the formation of primordia, with 70% of sawdust, being the first to form primordia and having the largest basidiome in diameter. It is believed that aeration is an important factor for mycelial and basidiome development in *P. ostreatoroseus*, contrary to what has already been mentioned for *P. djamor*, contributing to the differentiation of both species in cultivation situations. The mycelium of the species growing on yerba mate residue was able to reduce the inoculum of pathogenic fungi, indicating that it is an adequate practice for the treatment of the final residue. Thus, the yerba mate residue added with sawdust proved to be a possible substrate for the cultivation of *P. ostreatoroseus*, in addition to eliminating pathogenic fungi from plants.
Keywords: Cultivation; Alternative substrates; Shimeji; Mycelial growth.

Resumo
Cogumelos são cultiváveis em vários substratos de origem vegetal e o resíduo de erva-mate pode ser um dos mais promissores, especialmente se técnicas de cultivo domésticas forem desenvolvidas. Sendo um grande produtor e consumidor de erva-mate, o sul do Brasil também apresenta larga produção deste resíduo. Nesse sentido, este trabalho teve como objetivo testar o cultivo de *Pleurotus ostreatoroseus* no resíduo de erva-mate, buscando uma técnica para seu cultivo doméstico. Três substratos foram preparados: a) erva-mate parcialmente decomposta; b) erva-mate

recentemente descartada; c) erva-mate recentemente descartada adicionada de serragem de *Eucalyptus*. O cultivo de *P. ostreatoroseus* em erva-mate recentemente descartada e parcialmente decomposta mostrou-se inviável, com micelização do substrato, mas sem produção de basidiomas. No cultivo com serragem, todas as concentrações testadas apresentaram formação de primórdios, sendo a de 70% a primeira a formar primórdios e apresentar o maior basidioma em diâmetro. Acredita-se que a aeração é um fator importante para o desenvolvimento micelial e dos basidiomas em *P. ostreatoroseus*, ao contrário do que já foi mencionado para *P. djamor*, contribuindo para diferenciação destas. O micélio da espécie crescendo em resíduo de erva-mate também foi capaz de reduzir o inóculo de fungos patogênicos, indicando que é uma prática adequada para o tratamento do resíduo final. Dessa forma, o resíduo de erva-mate adicionado de serragem de *Eucalyptus* mostrou-se um substrato possível para o cultivo de *P. ostreatoroseus*, além de eliminar fungos patogênicos de plantas.
Palavras-chave: Cultivo; Substratos alternativos; Shimeji; Crescimento micelial.

Resumen
Los hongos son cultivables en varios substratos vegetales y los residuos de yerba mate pueden ser uno de los más prometedores, especialmente si se desarrollan técnicas de cultivo doméstico. Al ser un gran productor y consumidor de yerba mate, el sur de Brasil también presenta una gran producción de residuos. En este sentido, este trabajo tuvo como objetivo probar el cultivo de *Pleurotus ostreatoroseus* sobre residuos de yerba mate, buscando una técnica para el cultivo doméstico de este hongo. Se prepararon tres sustratos: a) yerba mate parcialmente descompuesta; b) yerba mate recién desechada; c) yerba mate recién desechada con la adición de aserrín de *Eucalyptus*. El cultivo de *P. ostreatoroseus* en yerba mate recientemente desechada y parcialmente descompuesta resultó inviable, con micelización del sustrato, pero sin producción de basidiomas. En el cultivo con serrín, todas las concentraciones presentaron formación de primordios, siendo el 70% la primera en formar primordios y en presentar el mayor basidioma en diámetro. Se cree que la aireación es un factor importante para el desarrollo del micelio y basidioma en *P. ostreatoroseus*, al contrario que la mencionado para *P. djamor*, contribuyendo a la diferenciación de estas especies. El micelio también fue capaz de reducir el inóculo de hongos patógenos, lo que indica que es una práctica adecuada para el tratamiento de los residuos finales. Así, los residuos de yerba mate añadidos con serrín de *Eucalyptus* demostraron ser un posible sustrato para el cultivo de *P. ostreatoroseus*, además de eliminar los hongos patógenos de plantas.
Palabras clave: Cultivo; Sustratos alternativos; Shimeji; Crecimiento micelial.

1. Introduction

"Yerba mate" or "erva-mate" (EM) is commonly consumed as an infusion produced from hot water extract (85°C to 95°C) of the dried leaves of *Ilex paraguariensis*, popularly known as "mate" or "chimarrão", or cold water as in the case of "tererê" (Arrieta, et al., 2018). More than 35 tons (ca. US$ 82 million) of yerba mate were exported by Brazil in 2016, with Uruguay, Chile, USA, and Germany being the main buyers. The per capita consumption of yerba mate in the Rio Grande do Sul (southern Brazil state) is 9 kg/year (ca. 70 thousand tons/year), with the state of Paraná consuming 20 thousand and Santa Catarina about 15 thousand tons (Ibramate, 2018).

The waste generated by consumption is simply discarded in the common garbage, being mainly directed to landfills or, on a smaller scale, to domestic fertilization. This lignocellulosic organic matter has been identified with many other alternative uses, including energy generation (Barreto & Menezes, 2015).

Yerba mate residue can also be used as the main base in the production of edible mushrooms, exempting the need for nutritional supplementation, as it presents an ideal C/N ratio. The residue is suggested as a base substrate, but only in the case of axenic production, since under normal conditions there would be a high rate of contamination due to the high nitrogen content (Reffatti, et al., 2006).

Taking into account that after use, the EM is entirely discarded and, as this is an organic waste in part with remains of branches, ribs, and leaves, it is possible that it is feasible to use this substrate for the production of mushrooms. The use of yerba mate as a substrate was tested and obtained positive results for *P. sajor-caju* (Kohari, et al., 1997). According to the authors, as it has a high level of nitrogen in its composition, yerba mate is a good substrate for mushroom production, since this element is essential for the basidiomata to develop.

The genus *Pleurotus* (Agaricomycetes – Basidiomycota) encompasses about 70 edible species and the main ones cultivated commercially are the following: *P. pulmonarius* (Fr.) Quél., *P. cystidiosus* O.K. Mill, *P. eryngii* (DC.) Quél. and *P. ostreatus* (Jacq.) P. Kumm. This group of mushrooms has high nutritional value, with several therapeutic properties and biotechnological applications (Rabuske, et al., 2019), and its production alone accounts for around 25% of those total cultivated mushrooms grown worldwide (Raman, et al., 2021). It is appreciated not only for its flavor but also for its nutritional and medicinal properties (Silva, et al., 2020).

P. ostreatoroseus Sing. is an interesting species for commercial use, due to its easy growth on various substrates without sterilization, with the possibility of growing at room temperature, and it presents a pink color, attractive to consumers (Putzke & Putzke, 2017). Due to its difficulty in being differentiated macroscopically, it is often confused with *P. djamor*. There are still no experiments using this species and this residue as substrate in literature.

The final EM substrate is characterized as one of the pure organic residues among those discarded in the domestic environment, since the use of pesticides is very controlled in production and the treatment in the industry uses only heat in processing and packaging. The possibility of cultivating mushrooms as an alternative for the final use of this noble substrate is then considered. This would make the mushroom produced of higher quality than produced using sawdust or other substrates.

The objective of this work was to evaluate the viability of the cultivation of *P. ostreatoroseus* for the first time in "erva-mate" residue in different formulations and to verify if the species controls pathogenic fungi found in this kind of waste.

2. Methodology

Inoculum collection

Experimental research was carried out (Pereira et al., 2018). The strains of *P. ostreatoroseus* (Pleurotaceae – Basidiomycota) was obtained from Brazilian commercial suppliers.

Substrate preparation

Three different compositions of yerba-mate (EM) substrates were prepared, discussed below, as follows:

1) EM partially decomposed; 2) Recently discarded EM; 3) Fresh EM with eucalyptus sawdust in three different concentrations (30%; 50%; 70%).

All different compositions were packed in glass containers (500 ml) and in bamboo stalks.

Experiment I:

Substrate 1: Effect of inoculation of *P. ostreatoroseus* on EM partially decomposed by filamentous fungi

The waste generated by the consumption of the plant in the form of "chimarrão" (prepared with hot water) was collected for 30 days, during the autumn and winter seasons of 2020, by five consumers, generating 150 winter and 150 autumn samples. These samples were placed in glass containers (500 ml) and remained at room temperature for two months, for the proliferation of fungi associated with decomposition. This naturally partially decomposed EM was used in the present work as a substrate for the production of *P. ostreatoroseus*.

To carry out the cultivation, 4 samples were chosen from the initial period of collection (1st, 2nd, 3rd, and 4th days), the other 4 samples from the intermediate period (13th, 14th, 15th, and 16th days), and 4 samples from the final period (27th, 28th, 29th and 30th days), this for both autumn and winter collections. These were placed in new glass containers (500 ml) and closed with a metal lid with a cotton pad inserted in a 5 mm hole in the center of it, to facilitate gas exchange with the medium (Curvetto, et al. 2002). The samples were sterilized in a pressure cooker at three different times (30, 60, and 90 minutes) (Prins & Paulsson, 2015). After cooling the substrate, each sample received a spoon of *P. ostreatoroseus* spawn, except for one

sample from each period, which served as a control. The samples were kept at room temperature under indirect light. 30 days after inoculation, the samples were evaluated for mycelial growth and were opened to assess the possibility of producing mushroom primordia.

Experiment II

Substrate 2: Fresh Yerba Mate (EM)

Fresh EM was tested as a substrate for the production of *P. ostreatoroseus*, using two forms of cultivation: non-sterilized and sterilized in a domestic pressure cooker (Prins & Paulsson, 2015).

For 25 days during the summer of 2021, the waste generated by the consumption of an EM was placed in a glass container (500 ml). Soon after being collected, the residue was sterilized in a pressure cooker for 30 minutes after being buffered. After cooling, a spoon of *P. ostreatoroseus* spawn was inoculated into the substrate. The glass was closed with a metal lid, with a central hole filled with a cotton swab, to facilitate gas exchange with the medium (Curvetto, et al. 2002). After complete micellization of the substrate, the glasses were opened for the production of primordia.

Another 20 glass recipients (500 ml) dipped in boiling water for 10 minutes received EM discarded directly, with a spoon of inoculum added to the surface of the substrate. The metal cap had a cotton swab inserted into a 5mm hole in the center.

The samples were kept at room temperature under indirect light. After full colonization of the substrate, the vials were opened and kept at room temperature for the production of mushrooms. A spray of sterile water was added daily after surface exposure.

Experiment III

Substrate 3: Fresh EM added with *Eucalyptus* sawdust

The residue of freshly discarded EM was added to 30%, 50%, and 70% (v/v) of dry eucalyptus sawdust (Kinge et al., 2016). The mixture was moistened to saturation and was placed in 500 ml glass containers (until full filling) and also in bamboo stalks 30 cm long (5 cm hole). Three glasses and three bamboos were prepared for each concentration. The glass samples were sterilized in a pressure cooker for 30 minutes, while the bamboo samples were not sterilized (Prins & Paulsson, 2015). After cooling, two spoons of *P. ostreatoroseus* spawn were added to the substrate of all preparations. The glasses were closed with a metal lid with a central hole filled with a cotton swab (Curvetto, et al. 2002). The bamboos were closed with a paper towel placed on the surface according to a methodology already published (Putzke et al., 2019).

The samples were placed under indirect light and room temperature for mycelial colonization of the substrate. With the total colonization of the mycelium, the lid was removed and the formation of the mushroom primordia was awaited.

Experiment IV

Effect of inoculation of *P. ostreatoroseus* on samples of EM with phytopathogenic fungi

In several samples, the occurrence of phytopathogenic fungi in the EM residue was detected (data still being prepared for publication). In these samples, the spawn of *P. ostreatoroseus* was inoculated. After total micellization of the substrate (30 days), an attempt was made to isolate the fungi previously found using a PDA culture medium. Inoculation was done in Petri dishes, in a solid medium, from fragments of the substrate and these dishes were kept at room temperature. The isolated fungi were identified using an Axiostar Plus microscope.

3. Results

Experiment I

Effect of inoculation of *P. ostreatoroseus* on EM substrate partially decomposed by filamentous fungi

After one month of inoculation of *P. ostreatoroseus* on yerba mate (EM) substrate partially decomposed by other fungi, the samples were evaluated for mycelial growth. The mycelial growth on this substrate did not result in a good development, as described for the species in other media. Samples collected in winter had average substrate colonization of 5.5% and samples selected from the autumn season had an average of substrate colonization a little higher, of 16%.

Therefore, mycelial colonization by *P. ostreatoroseus* in EM substrate partially decomposed by filamentous fungi, even if autoclaved before inoculation, was not satisfactory. However, there was a difference between the winter and autumn seasons, in which the samples colonized in autumn had a higher average of micellization. This difference in the colonization of the substrate may be due to the maturing time of the yerba mate at the time the spawn was inoculated since the winter season samples were 3 months older than the autumn season ones. Another reason why this colonization of the substrate by the edible fungus was so low could also be since the EM has already been completely degraded by the filamentous fungi, making it an unsuitable substrate for *Pleurotus*.

Thus, it was possible to see that EM with a shedding time of more than two months and previously colonized by other fungi cannot be used as substrate in the production of edible mushrooms *P. ostreatoroseus*.

Experiment II

Effect of direct inoculation *P. ostreatoroseus* on fresh discarded EM

The 25 glasses of fresh unautoclaved EM substrate had full colonization of the medium in all samples by 7 days, whereas the 20 flasks with fresh autoclaved EM substrate took 17 days for full colonization. However, even though the mycelial colonization of the substrate was complete, the formation of mushroom primordia after opening and moistening the surface was not observed in both, with subsequent degeneration of the mycelium occurring.

Experiment III

Effect of inoculation of *P. ostreatoroseus* in EM substrate added with eucalyptus sawdust

All glass flasks had total colonization of the substrate by the mycelium of *P. ostreatoroseus* in 30 days, being then opened for exposure and formation of primordia, being humidified for 9 days. After this period, the primordia emerged from EM.

The first substrate in which there was formation of primordia was that of a 70% concentration of eucalyptus sawdust. The formation took place nine days after opening the glass and moistening the substrate. Subsequently, there was also formation of primordia in the substrate of 50% of sawdust and finally in the concentration of 30% of sawdust. The effect of the addition of sawdust should be mainly on the maintenance of aeration in the substrate, which is difficult to obtain with pure EM.

In bamboo containers, it was not possible to evaluate mycelial colonization by the opacity of the material. However, after 9 days, there was formation of primordia in one of the samples, in which the concentration of eucalyptus sawdust in the substrate was 30%. The technique, therefore, proved to be adequate, but due to excessive humidification, the final result for most samples was not positive.

Experiment IV

Effect of inoculation of *P. ostreatoroseus* on EM samples with phytopathogenic fungi

Flasks samples with the detection of phytopathogens that were inoculated in PDA, after the mycelial growth of *P. ostreatoroseus*, all had negative results, with no isolation of any of the species originally found. This demonstrates that the edible mushroom species can eliminate yerba mate phytopathogenic fungi and leave the substrate improved for disposal purposes.

4. Discussion

Refatti, et al. (2006) mention that EM residues can be used as the main basis for the formulation of the substrate in the production of *Pleurotus* spp., without the need for supplementation. However, the results found in this work for *P. ostreatoroseus* demonstrate the opposite. Although there is complete colonization of the substrate formed solely by discarded EM, there is no formation of basidiome, indicating that there is a need for supplementation of the substrate by some other substances or modification of its physical properties Thus, it is believed that the pure EM substrate does not support the formation of mushroom primordia, because the aeration of the substrate, necessary for the development of them was not sufficient, since compaction of the medium was perceived and also reported by other authors (Maddau, et al., 2002). Eventually, for other species of *Pleurotus*, this characteristic may be adequate, as suggested for *P. sajor-caju* (Kohari, et al., 1997).

It is known that the mycelial colonization of some *Pleurotus* species depends on good aeration (O2/CO2 ratio), and transpirant bags have even been developed to accelerate the process in *P. eryngii* (Maddau, et al., 2002). In "in vitro" studies with sealed Petri dishes, the species *P. sajor-caju*, *P.pulmonarius*, and *P. florida* had no significant differences in growth in aerobic and anaerobic conditions (Tolentino, 2016). For *P. djamor*, a species sometimes considered the same as *P. ostreatoroseus*, there were also no differences in growth under aeration or with sealed plates (Bumanlag, et al., 2018). This characteristic can also be used to differentiate both species under growing conditions.

It is known that *Pleurotus* fungi are more aggressive in competition with microorganisms, so much so that the substrate would not even need to be sterilized before inoculation (Chang & Quimio, 1982; Sanchez, 2010). Cultivated fungi present a series of metabolism products capable of reacting with other contaminating fungi, including plant pathogens, in order to successfully colonize a substrate (Money, 2016). *Pleurotus* spp. control filamentous fungi and nematodes during their growth, and their role as biological controllers is still underestimated (Putzke & Putzke, 2019).

Several works mention the possibility of using in animal feed the final residue from the production of edible mushrooms of the genus *Pleurotus*, creating a closed cycle at the end of production and without generating residues. Calves supplemented with 50% of the microbially fermented mushroom substrates used had a trend of 8-12% increased live weight gain compared to the control group (Kim, et al., 2012). Rice straw-based compost used in mushroom production can replace 75% of fresh Napier (MS) grass for cattle feed (Suwandyastuti & Bata, 2012). More studies have revealed applications as food for birds and fish, among others (Foluke, et al., 2014; Sartori, et al., 2015).

5. Conclusion

The EM residue added with eucalyptus sawdust proved to be suitable for the production of edible mushrooms *P. ostreatoroseus*. The procedure tested here can be applied to the domestic environment and yerba mate consumers can locally produce their mushrooms for consumption. The EM is one of the substrates with less toxic substances, resulting in a much healthier mushroom. The recommendation for the use of this substrate can also be applied to commercial producers. The

inoculated fungus eliminates the yerba mate pathogens present and viable in the substrate, which could even infect other cultures, resulting in an innocuous disposal material. It is important to highlight that several studies mention the possibility of using the final residue from the production of edible mushrooms in animal feed diminishing the waste production.

Further research may be useful in finding even more efficient addition formulas to the yerba mate substrate. Furthermore, since yerba mate added to eucalyptus sawdust proved to be a good substrate for the cultivation of *P. ostreatoroseus*, it may also be a good substrate for other species of the same genus, which deserves to be investigated in future research.

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