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Cultivation of *Pleurotus ostreatus*, *Macrocybe titans* and C*oprinus comatus* from Composted Household Organic Wastes of Yerba Mate (*Ilex paraguariensis*) and Coffee Grounds

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HIGHLIGHTS

- A 1:1 ratio of used yerba mate and coffee grounds proves to be an effective compost for mushroom cultivation.
- Notable biological efficiency achieved with *Coprinus comatus* on compost.
- Cultivation bag size does not significantly affect *C. comatus* basidiomata size.
- *Macrocybe titans* produced smaller-than-expected basidiomata in confined bag cultures.

Abstract: Large quantities of coffee and yerba mate are consumed in households in Argentina and Brazil. In this study, we investigated the growth and production of *Coprinus comatus*, *Macrocybe titans* and *Pleurotus ostreatus* on compost of used yerba mate and coffee grounds to determine if these household wastes are suitable for the cultivation of edible mushrooms.
Prior to cultivation, a virtual survey was conducted and completed by 156 household representatives of households in Argentina, confirming the initial premise that Argentinians consume large amounts of yerba mate and coffee, resulting in significant quantities of these household wastes that could be reused for the cultivation of edible mushrooms. The initial composition of the compost was formulated with a 1:1 ratio of used yerba mate and coffee grounds, with a humidity level of 65%. The compost was maintained for 26 days and was turned twice per week. Several composts were made to observe the variability of the results. For *P. ostreatus*, a treatment was included where the initial substrate was used without composting. Blonde peat served as casing layer for the soil species, and they were placed in a production chamber at 20°C, photoperiod, 85-90% relative humidity, and aeration. The harvested basidiomata were weighed and the biological efficiency (BE) was calculated. While the efficiencies of *Pleurotus ostreatus* and *Macrocybe titans* were not outstanding, those obtained with *C. comatus* ranged from 58.7% to 177.3%. *P. ostreatus* and *M. titans* produced basidiomata of smaller size than nature, but *Coprinus comatus* generated basidiomata of equal or larger size.

Keywords: Agaricales; Alternative substrates; Compost; Mushroom; Production.

INTRODUCTION

The composition of urban waste varies based on regional factors, economic activities, purchasing power and cultural habits of consumers. In Argentina, it has been estimated that the volume of urban solid waste sent to CEAMSE (Coordinación Ecológica Área Metropolitana Sociedad del Estado), which includes municipalities in the Buenos Aires Metropolitan Area (AMBA) and others in the province of Buenos Aires, represents a third of the country's total. It has reported that the total incoming municipal solid waste in 2023 was 5,753,253 tons [1], while in 2022 it was 5,753,253 tons with 41.64% of food waste [2]. In Brazil, it is estimated that approximately 77.1 million tons of urban organic waste were generated in the country in 2022 [3] and it is estimated that 45.3% corresponds to organic waste [4]. A portion of this organic waste could potentially be repurposed for the cultivation of edible mushrooms, given that fungi naturally degrade organic matter as part of their metabolic processes. This motivates an exploration of the extent to which such waste can be utilized for mushroom production, concurrently contributing to a reduction in overall waste production.

The consumption pattern of yerba mate (*Ilex paraguariensis* A.St.-Hil) in Argentina is particularly significant, with an estimated average per capita consumption of 5.63 kg in 2024. This value was calculated based on the total domestic market consumption of 258,813 tons [5] and the national population for that year [6]. In Brazil, yerba mate production reached 736,893 tons in 2023 [7]. The state of Rio Grande do Sul stands out for being Brazil's largest producer, consumer and exporter of yerba mate, with a per capita consumption of more than 9 kg/person/year [8]. Moreover, Brazil is the largest coffee producer, with a production of 66.4 million of 60 kg bags per year during 2024–2025 [9]. Brazil's domestic consumption is 21 million bags per year, equivalent to 5.01 kg of roasted coffee per capita per year and 6.26 kg of green coffee per capita per year in 2024 [10]. These consumption patterns suggest the substantial production of yerba mate and coffee ground residues in Argentina and Brazil, raising the prospect of their potential utilization in mushroom cultivation.

Edible mushrooms are recognized for their exceptional nutritional value [11-14] and have been successfully cultivated on organic residues, capitalizing on their unique properties [15,16]. This cultivation method not only circumvents the need for fertile land but also minimizes spatial requirements.

Within the global macromycete industry, wild mushrooms, along with cultivated edible and medicinal mushrooms, constitute three major components. In 2013, the industry was valued at USD 63 billion, with cultivated edible mushrooms contributing 54% (approximately USD 34 billion), medicinal mushrooms 38% (USD 24 billion), and wild mushrooms accounting for USD 5 billion, equivalent to 8% of the total [17]. In Argentina, a survey estimates that the most recognized species is *A. bisporus*, followed *by Pleurotus* spp. [18].

The genus *Pleurotus* (Fr.) P. Kumm is renowned for its numerous edible species with excellent flavor and texture. Its global popularity has surged in recent years due to its adaptability to a wide range of temperatures and its capacity to thrive on various lignin- and cellulose-rich substrates [19,20]. The environmental benefits of cultivating *Pleurotus* species include the utilization of materials derived from agricultural and agroindustrial activities, promoting effective organic waste management [21]. A literature summary, highlighted the biological efficiencies (BEs) of different substrates on *Pleurotus* cultivation. Coffee pulp substrate, for instance, demonstrated BEs ranging from 67.1% to 225.2% for various species, including *P. ostreatus*, *P. sajor-caju*, *P. pulmonarius*, and *P. djamor* [22]. Notably, bags formulated with 100% coffee pulp achieved a BE of 168% for *P. ostreatus* [23]. However, it was observed that strains took longer to colonize coffee pulp, and bags with this substrate required more time to produce fruiting bodies compared to those with substrates containing coconut and cocoa husks.

Coprinus comatus (O. F. Müll.) Pers. is a soil fungus with edible basidiomata that self-degrade rapidly. Despite this, it is of great interest due to its detected antitumor properties [24], blood glucose-lowering effects [25,26], and antioxidant properties [27,28]. In the literature, composted wheat straw enriched with horse and/or chicken manure, adjusted to 70% humidity, is mentioned as a substrate for the production of basidiomes [29].

Macrocybe titans (H. E. Bigelow & Kimbr.) Pegler, Lodge & Nakasone is a soil fungus endemic to the Americas, particularly tropical areas. In recent years, samples have been discovered in Argentina, specifically in the province of Corrientes [30]. This species produces large, fleshy basidiomata, with the pileus measuring 30 to 100 cm in diameter [31], and it has been reported to have a pleasant aroma and good flavor of the flesh of the basidiomata of this species [32]. The limited literature on its cultivation method makes it a species of interest for cultivation, especially given its production of large basidiomata. It is an edible species of particular interest due to the remarkable size of its basidiomata and the scarcity of information on how to cultivate it, which makes it especially relevant to explore new cultivation strategies. In addition, it has been discovered that *M. titans* produces polysaccharides in its basidiomata that prevent the migration of cancer cells [33]. These data show that it is a fungus with great potential both as an edible and medicinal mushroom.

The objective of this study is to evaluate the cultivation of strains of *Coprinus comatus*, *Macrocybe titans*, and *Pleurotus ostreatus* (Jacq.) P. Kumm. on compost made with spent yerba mate and coffee grounds (including an unused compost assay for *P. ostreatus*), analyzing basidiome production, morphological parameters, and compost characteristics.

MATERIAL AND METHODS

Survey

Before starting the study, an online survey was conducted using a digital questionnaire by 156 Argentine households to obtain information on yerba mate and coffee consumption, as well as interest in mushroom cultivation. The questionnaire included general questions about age and place of residence. It also addressed yerba mate and coffee consumption, including frequency, quantity, daily intake, and monthly household consumption in kilograms. Finally, it asked whether respondents would consider growing edible mushrooms at home if provided with the necessary information and resources.

Strains

The following strains deposited in the Agaric Mushroom Ceparium CEHA of the Faculty of Exact and Natural Sciences, University of Buenos Aires, Argentina, were used:

Coprinus comatus: CEHA 104, collected from soil in the province of Buenos Aires, Argentina, leg. Aimone D. P., 25-XI-2018.

Macrocybe titans CEHA 105, found in soil in the Paraná delta, Buenos Aires, Argentina, 9-XII-2018.

Pleurotus ostreatus CEHA 95, strain isolated on dead wood of *Acacia melanoxylon*, in Ciudad Universitaria, Buenos Aires city, Argentina,15-VI-2018.

Substrate

Used yerba mate (*Ilex paraguariensis*) and coffee grounds, obtained from household consumption, were collected, stove-dried at 50ºC during two days separately, and stored in a cold room at 6°C for 3 months. The initial substrate was prepared by mixing the dried yerba mate waste and coffee grounds in a 1:1 ratio in a 45-liter plastic container. Water was added until it reached 63% humidity (field capacity of the substrate), and the mixture was kept for 26 days in a room maintained at 24°C. However, the compost temperature was observed to be higher than the ambient temperature. The substrate was mixed twice a week. Polypropylene bags filled with 180 g of compost and 6 g of $CaCO_3$ were sterilized at 121°C for 2 hours in an autoclave.

Three replicates of compost (compost 1, compost 2 and compost 3) were made to assess the variability and reproducibility of the results. Additionally, for each compost, 3 to 7 bags of 0.2 kg per species were made based on the amount of initial substrate. The number of replications was confirmed by Rabinovich's test [34]

For *P. ostreatus*, an additional treatment was used: the initial substrate of 50% yerba mate and 50% non-composted coffee to compare the results with those of the composted substrate.

Compost characteristics

After composting, a portion was taken to measure moisture, pH and conductivity. Moisture was determined by weighing 20 g of wet compost, drying it in an 80°C oven for 24 hours, and calculating the moisture percentage. The pH and conductivity were measured by hydrating 10 g of wet compost with 50 ml of distilled water, stirring for 5 minutes, and using a pH meter (HI 2221 Calibration Check pH/ORP Meter, HANNA Instruments) and a conductivity meter (Pocket Pal, TDS Tester) calibrated with 0.01M KCl solution.

Inoculation and incubation of the bags

Each bag was inoculated with 14 grams (7% w/w) of candeal wheat seed with mycelium. Bags of *C. comatus* and *P. ostreatus*, were incubated in dark chamber at 24°C for 2 weeks [35], while *M. titans* was incubated at 28°C [36].

Basidiome production

For *C. comatus* and *M. titans*, casing layer was prepared using blond peat from Tierra del Fuego *Sphagnum* moss. The peat was hydrated in $Ca(OH)_2$ water to achieve a pH 7. Then, it was placed in polypropylene bags and maintained at 60°C for 2 hours in an oven [37].

Culture bags were left overnight in a cold chamber at 6°C to induce heat shock. The block formed by the mycelium and substrate was removed from the culture bags and transferred to plastic containers sterilized with alcohol. Fifty grams of casing layer were added to the walls of the colonized block, which was then placed in a culture chamber at 20°C [35] with a photoperiod of 12 hours of light (400-500 lux) and 12 hours of darkness [38]. The culture chamber maintained a relative humidity of 85-90%, with air renewal six times per hour, and sprinkler irrigation once a day. For *P. ostreatus*, the bags were opened, and they were hung in the same culture chamber under identical conditions.

The basidiomes produced were collected, weighed, and the biological efficiency (BE) of each bag was calculated by summing the weights of all harvested basidiomes using the formula: Biological efficiency= (Fresh weight of basidiomata/dry weight of compost) x 100%.

Basidiome measurements

In all three species, the length and width of the foot were measured. However, different considerations were taken for the pileus, due to the distinct shapes of each species. For *C. comatus*, with a cylindrical to campanulate pileus, two measurements were made: one for the height of the pileus, from the ring to the tip, and another for the width perpendicular to the length where it had the greatest measurement. For *P. ostreatus*, with a demediate or semicircular shape in an apical view, the length was measured from where the lateral foot ends to the opposite edge, and the width was measured perpendicular to the length along the longest distance line. In *M. titans*, the diameter of the pileus was measured, given its practically isodiametric nature [39].

Statistical study

Statistical analysis was performed using an ANOVA with Bonferroni test to compare the variability between composts and among basidiome size variables [40]. Assumptions of normality were verified using the Shapiro-Wilks test, and homogeneity of variances was assessed with the Levene test [41]. Variables that did not meet any assumption underwent Log10 transformation or variance modeling was conducted in R and R-studio with the {NLME} statistical package. All tests were performed with the criterion defining statistical significance as type 1, $p<0.05$.

RESULTS AND DISCUSSION

Survey

Of the respondents of the virtual survey, 50.6% were located in the province of Buenos Aires, 38.5% in the Autonomous City of Buenos Aires, and 10.9% in other provinces of the country. Analysis of the survey data revealed that 72.4% of the total respondents were yerba mate consumers, with half of them consuming yerba mate between two to three times per day. Among households that included yerba mate in their consumption habits, a substantial 77% reported consuming 1 kg or more per month. Additionally, the survey indicated that 91% of households consumed coffee, with 45.5% expressing a preference for filter coffee, thereby contributing to the generation of coffee grounds. More than half of the coffee consumers reported drinking coffee 1 or 2 times a day. These findings underscore the significant monthly consumption of yerba mate and coffee in Argentine households, resulting in the generation of substancial organic waste. This observation supports the notion that yerba mate and coffee grounds were promising options for home mushroom cultivation. The decision to exclude tea waste from consideration was based on its comparatively lower consumption in households compared to yerba mate.

In terms of interest in growing edible mushrooms, only a marginal 8.3% of respondents expressed no interest in such cultivation. Notably, 34% conveyed a potential interest, indicating a willingness to consider mushroom cultivation if given the opportunity. Most respondents, comprising 57.7%, expressed a strong inclination to grow edible mushrooms. These findings suggest a notable receptivity among survey participants towards the cultivation of edible mushrooms at home. For more information about the survey, see the link to the data repository in the supplementary material.

Compost characteristics

Yerba mate biomass contains between 3.3% and 4.2% total nitrogen [42,43], while nitrogen compounds in coffee display relatively stable concentrations across species and remain consistent even after the roasting process, with values ranging from 8.5% to 13.6% [44]. However, these data refer to unused yerba mate and coffee, so the nitrogen content may decrease after use. For example, coffee grounds contain approximately

2% nitrogen [45]. According to Stamets and Chilton [29], compost formulations should initially contain between 1.5% and 1.7% nitrogen. Therefore, a 1:1 mixture of used yerba mate and coffee grounds would result in a nitrogen content slightly above the level recommended by these authors.

The pH of the different composts (Table 1) was slightly higher than the recommended range [46], but did not change significantly, as the medium tends to acidify during fungal growth. As shown in Table 1, the moisture content was also slightly higher than recommended [46], while electrical conductivity remained between 446 and 719 µS/cm.

Table 1. Characterization of the different composts used on crops.

	Compost 1	Compost 2	Compost 3
% Humidity	70.1± 0.5	74.2 ± 3.5	74.9 ± 1.8
pH (26 °C)	7.7 ± 0.1	7.4 ± 0.1	7.9 ± 0.2
Conductivity (µS/cm)	446 ± 0	582 ± 37	719 ± 25

The characteristics of the compost obtained fall within the acceptable ranges for high-quality compost according to Deschamps [46], as its color, odor, electrical conductivity, and the possible presence of actinobacteria—indicated by the smell of geosmin—are within the expected parameters. However, both the pH and moisture content were slightly higher than optimal.

Several studies on the cultivation of *Agaricus bisporus* suggest that the electrical conductivity of the casing layer must be low (typically below 1100 µS/cm), as higher conductivity is associated with reduced basidiomata yield and delayed primordia formation [13,47]. Considering these findings, compost made from yerba mate and coffee grounds in a 1:1 ratio could be evaluated for use in casing layer studies, since its electrical conductivity remained below 800 µS/cm.

Basidiomata production

In Figure 1, some of the basidiomes obtained during the trials can be observed.

Figure 1. Basidiomata produced in yerba mate and coffee grounds compost. A and B. *Macrocybe titans.* C. *Coprinus comatus.* D. *Pleurotus ostreatus.*

M. titans took approximately one month to produce the first harvest. Therefore, to enable a valid comparison among species, only the first harvest was considered, as it is typically the most productive.

Trials with non-composted substrate were not conducted for *C. comatus* and *M. titans* (Table 2), given their growth habit in soil [32,48] and the limited information on their cultivation from wild strains, unlike *A. bisporus* [49]. This differs from *Pleurotus*, which has shown greater versatility in substrates [19,20].

The BEs obtained for *C. comatus* ranged between 88% and 145%, with no significant differences observed among the different compost treatments (Table 2). Dulay and coauthors [50] cultivated *C. comatus* on paper waste and, although substrate colonization was rapid, they reported biological efficiencies below 24%. In another study, Dulay and coauthors [51] used residual substrate from *Pleurotus* cultivation, obtaining biological efficiencies lower than 10%.
Yerba mate and coffee have an initial pH of approximately 4.2 [43,45]. However, in the composts used in this study, pH levels increased to between 7.3 and 8.1 (Table 1), which are more suitable for fungal cultivation, particularly for species such as *A. bisporus* and *C. comatus* [29,37,46]. This shift in pH may partially explain the good performance of C. comatus observed in this compost (Table 2).

Table 2. Biological efficiency with its standard error of the different species according to substrate. ANOVA tests with Bonferroni and Tukey analysis were performed to compare substrates in *P. ostreatus* and *C. comatus* with a significance level of 95%. *N.S.= not studied.

Substrate	Biological efficiency (%)		
	Pleurotus ostreatus	*Coprinus comatus*	*Macrocybe titans*
Compost 1	52.5 ± 5.5 a	145.0 ± 32.3 A	46.7
Compost 2	N.S.	88.1 ± 29.4 A	13.5 ± 3.2
Compost 3	30.0 ± 3.0 b	101.7 ± 37.8 A	48.3 ± 22.2
Non-composted	50.5 ± 11.1 a	N.S.	N.S.

When comparing the biological efficiencies obtained for *P. ostreatus* (Table 2), significant differences were observed between composts, and between composted and non-composted substrates, unlike *C. comatus* which showed no significant differences. This may indicate that *P. ostreatus* is more sensitive to substrate variation than *C. comatus*. ANOVA tests were not conducted for *M. titans* as the replicate number was not adequate, because the number of contaminated bags was high, leaving only a total of three bags per compost.

In *Pleurotus ostreatoroseus*, Klotz-Neves and coauthors [52] observed low mycelial growth on partially decomposed yerba mate, while freshly discarded yerba mate supported mycelial growth but not primordium formation. The authors also tested mixtures of eucalyptus sawdust with fresh yerba mate and obtained primordium formation, although they did not report the biological efficiency (BE) of these treatments. Kohari [53] reported a BE of 21.1% for *P. ostreatus* on substrates containing yerba mate; in that study, the substrate was not previously fermented, as suggested by Guzmán and coauthors [21].

In our study, no significant differences in BE were found between compost 1 and the non-composted treatment. However, both differed significantly from compost 3 (Table 2). Good mycelial growth was observed, with complete colonization of the bag in approximately two weeks, followed by basidiome formation. It can be inferred that the addition of coffee grounds may have been one of the factors contributing to the improved results.

The same *P. ostreatus* strain used in this trial produced a BE of 67% on coffee grounds [54]. Additionally, two strains of *Pleurotus* spp. grown on coffee pulp yielded BE values ranging from 57.7 to 59.4% [55]. Piña-Guzmán and coauthors [22] reported BE values ranging from 67.1 to 225.2% on coffee pulp for *P. ostreatus*, *P. sajor-caju*, *P. pulmonarius*, and *P. djamor*. Similarly, García-Oduardo and coauthors [23] documented a BE of 168% for *P. ostreatus* grown on a substrate composed entirely of coffee pulp.

These findings suggest that cultivating *P. ostreatus* directly on spent coffee grounds or coffee pulp, without the addition of yerba mate, may yield better results. Although the BE obtained in this study using a yerba mate/coffee compost was lower than those reported in the literature, it is important to note that only a single flush was harvested, which may have limited the total yield.

In certain cases, substrate fermentation has been reported to increase nitrogen availability [56]. This could suggest that the lower BE of *P. ostreatus* observed on composted substrates may be related to increased nitrogen availability in the medium, potentially favoring vegetative mycelial growth over basidiomata production.

The BEs obtained with *M. titans* were good (see Table 2) when compared with the 59.26% BE reported for *Macrocybe crassa* by Inyod and coauthors [57]. The only study found on the cultivation of *M. titans* is by Duong and coauthors [38], who obtained yields ranging from 12.5 to 14.2% (150 to 175 g per culture bag of 1.2 to 1.4 kg fresh substrate) using wheat straw and rubber sawdust, both supplemented with rice bran. In the present study, similar yields were obtained, ranging from 2.66 to 17.3% (17.3% in compost 1, 3.5 ± 0.8% in compost 2, and 12.1 ± 4.5% in compost 3).

Macrocybe titans has significant potential due to its large basidiomata, with a pileus that can measure 8.5 to 25 cm in diameter and stems between 12 and 27 cm in its natural habitat [39], or up to 30 to 100 cm in diameter as reported by Pegler and coauthors [31]. In this work, basidiomata obtained on compost were smaller, with pilei measuring 3 to 6 cm in diameter and stems 2.2 to 8 cm in length and 1 to 2 cm in width (Figure 2), much smaller than those found in nature [32]. This may be attributed to the small size of the bags, as the development from primordium to basidiomata took approximately 12 days.

As shown in Figure 2, the basidiomata sizes of *C. comatus* and *M. titans* do not exhibit significant differences between composts. The pilei of *C. comatus* measured 2 to 15 cm in length and 1 to 4 cm in width, while stem lengths ranged from 7 to 30 cm with widths of 0.5 to 3 cm. These measurements are consistent with the literature, which reports pilei of 3 to 5 cm in diameter and 4 to 15 cm in height, and stem sizes of 6 to 15 cm in length and 1 to 2 cm in width [48]. The only notable difference is the longer stem length in culture (7 to 30 cm), which could be due to cultivation conditions, where light and CO_2 concentration influence the production of basidiomata with longer stipes [35].

In *P. ostreatus*, significant differences in basidiomata size were observed between composted and non-composted substrates, with the latter producing significantly ($p \leq 0.05$) larger basidiomata. However, no significant differences were found between the different composts ($p > 0.05$). Lechner and coauthors [58] reported that *P. ostreatus* presents pilei measuring 110 x 130 mm and stipes of 15–20 x 11–15 mm on substrates with 200 g dry weight, larger than those obtained in this study.

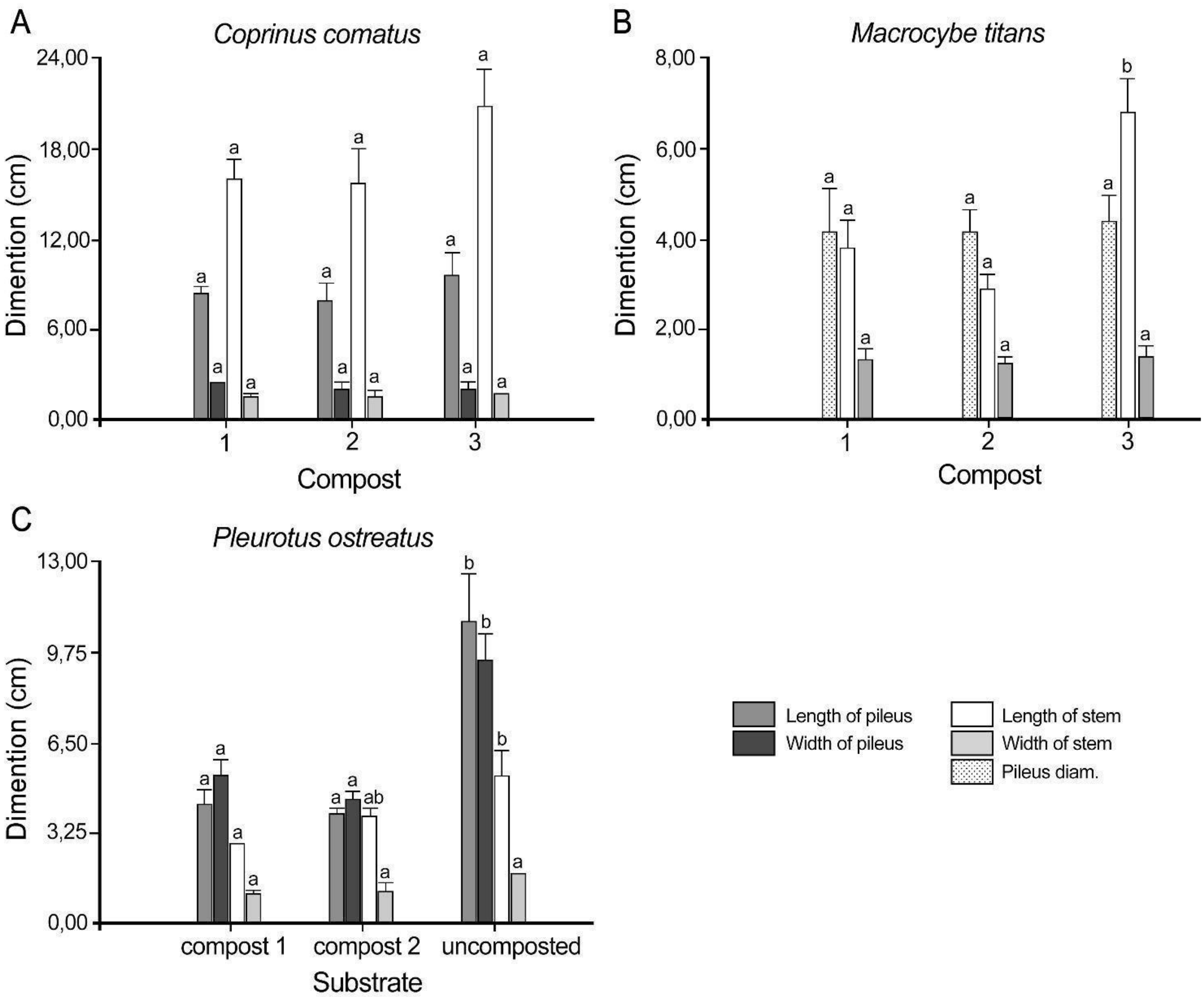

Figure 2. Basidiome measurements with 95% confidence interval. **A.** *C. comatus.* **B.** *M. titans.* **C.** *P. ostreatus*.

The objective of this work was to produce these mushroom species on substrates from household waste and sterilized for this purpose, however there are several methods of pasteurization by heat [29,59,60] or chemical [61] that can be used for future experiments that would bring them closer to a family production.

CONCLUSION

The use of a 1:1 ratio of used yerba mate and coffee grounds generated a compost suitable for the cultivation of the species studied, emerging as a promising substrate, particularly for *C. comatus*, which achieved notable biological efficiency compared to previous results for the cultivation of the same species. It is also interesting to note that these biological efficiencies are comparable to those obtained with well-established edible mushroom species.

Considering the limited amount of literature on the cultivation of *Macrocybe titans*, the results obtained in this study represent a valuable contribution to the cultivation of this edible mushroom, which has significant potential due to its large basidiomata in nature. The smaller basidiomata observed in this study may be attributed to the size of the culture bags.

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Conflicts of Interest: The authors declare no conflict of interest.

Data Availability Statement: Research data are only available upon request for corresponding author.

Supplementary Material

Survey on yerba mate and coffee consumption, and home composting in Argentina can be checked on https://ri.conicet.gov.ar/handle/11336/273629 [62].

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