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Effect of Culture Media on the Mycelial Growth of Two Edible Mushrooms, *Pleurotus ostreatus* (Jacquin) Kummer and *Marasmiellus inoderma* (Berk.) Singer

Dania, A.T.* [1,2] and Dania, B.B.[3]

[1]Department of Plant Biology and Biotechnology, University of Benin.
[2]African Centre for Mushroom Research and Technology Innovations (ACMRTI), University of Benin.
[3]Department of Science Laboratory Technology, University of Benin.

*Corresponding author Email: theophilus.dania@uniben.edu, Tel: +2348066696469

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ABSTRACT: This study evaluated the effects of different synthetic and formulated culture media on the mycelial growth of *Pleurotus ostreatus* and *Marasmiellus inoderma* under standard laboratory conditions. The synthetic media included Potato Dextrose Agar (PDA), Yeast Extract Agar (YEA), Malt Extract Agar (MEA), and Czapek-Dox agar, while formulated media in are cornmeal, rice extract, and wheat extract agars. Mycelial plugs were inoculated into each medium and incubated, with growth monitored daily. However, after five days of inoculation, the highest mycelial growth was recorded for PDA (71.67 ± 3.3 mm) and cornmeal (71.33 ± 1.3 mm), followed by malt extract agar (67.50 ± 2.5 mm). *Marasmiellus inoderma* exhibited faster mycelial extension than *Pleurotus ostreatus*, recording the highest growth rate in MEA (27.99 ± 0.58 mm/day) and the lowest in rice extract agar (23.61 ± 0.90 mm/day). *P. ostreatus* showed peak growth in PDA (14.33 ± 0.65 mm/day) and the least in rice extract agar (11.43 ± 1.12 mm/day). Biomass accumulation and mycelial density followed similar trend, with *M. inoderma* producing the highest dry weight in MEA and *P. ostreatus* in PDA. These results demonstrate that both species can utilize various nutrient sources and that low-cost, locally formulated media offer comparable alternatives to synthetic media for sustainable mushroom cultivation.

Keywords: *Marasmiellus inoderma*, Mushroom, Mycelial, *Pleurotus ostreatus*

Introduction

Mushrooms have become well-known globally for their nutritional and therapeutic properties. Cultivating them, serves as a beneficial bioconversion method, transforming waste materials into potentially valuable resources (Subebi *et al* 2024). Mushroom cultivation fits in very well with conversion of crop residue into valuable food protein and is considered as potential source of income, alternative food production, provision of employment as well as for recycling of agricultural wastes. (Aditya *et al*., 2024). A Mushroom is generally defined as a macro fungus that has a visible fruiting body, which either grows above or below the soil, and is large enough to be seen without optical assistance and plucked up with the human hands for different uses (Chang and Miles, 1992). A mushroom is the fleshy, spore-bearing fruit body of a fungus, typically produced above ground or on a substrate (Boa, 2004; Oei, 2003). In comparison to other plant and animal protein sources, mushroom proteins consist of all essential amino acids, hence have high biological efficiency and economic importance as the mushrooms can grow and produce food in a short time compared to other plant-based protein sources (González *et al.*, 2020). They have a wide range of ecological adaptation as well as the ability to transform natural wastes into edible biomass.

Mushroom growth is highly influenced by several factors such as spawn, growing media, pH, temperature, moisture content and light intensity. In mushroom cultivation spawn production is one of the major limiting factors to all over the world (Stanley and Waadu, 2010). The growing medium is the primary factor that holds

the utmost significance in mushroom production. This medium provides vital nutrients essential for the development of mycelium. When the medium is nutritionally enriched, it facilitates abundant growth of the mycelium by fulfilling all the nutritional requirements and creating optimal physical conditions for vegetative growth (Aditya *et al*., 2024). The success or failure of mushroom cultivation largely relies on a thorough and accurate comprehension of their nutritional and environmental needs. Studies with comparative analysis of the effects of commercial culture media and natural media from the local wastes on fungal growth deserve the special attention (Rizal *et al*. 2016, Landingin *et al.*, 2020, Arana-Gabriel *et al*., 2020, Dulay *et al*,. 2021).

Oyster mushrooms, scientifically known as *Pleurotus* species, belong to the class Agaricomycetes, the order Agaricales and the family Pleurotaceae. They are commonly found in temperate and tropical forests, occurring naturally on decomposing logs or occasionally on dried trunks of both deciduous and coniferous trees. They have a wide range of ecological adaptation also have the ability to transform natural wastes into edible biomass. *Pleurotus ostreatus* is a type of oyster mushroom that is edible. It is the second most cultivated edible mushroom in the world because it has high nutritional and economic value, and also high medicinal properties

Marasmiellus inoderma is an edible mushroom picked from the wild and eaten by some tribes in Nigeria (Nicholson, 1989). De Kesel et al. (2002), reported the growth of M. *inoderma* on oil palm waste. The aim of this study is to evaluate the effects of different culture media, both synthetic and formulated, on the vegetative growth of *Pleurotus ostreatus* and *Marasmiellus inoderma* under laboratory conditions.

Materials and methods

Pure cultures of *M. inoderma* and *P. ostreatus* were collected from the African Centre for Mushroom Research and Technology Innovations of the University of Benin. The cultures were sub-cultured and kept in the incubator at 4 ℃ for further use in the study. Broth from rice, and wheat grains were used to prepare formulated culture media rice dextrose agar (RCEA), wheat dextrose agar (WHT).

Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Czapek-Dox, Cornmeal media were prepared using standard methods. After sterilization, the media were removed from the autoclave and allowed to cool to about 45 ℃. The media was then dispensed into disposable Petri-dishes of 85mm in diameter and allowed to cool and solidify in the Laminar flow cabinet. Mycelial discs (5 mm) from a 7-day old culture of *M. inoderma* and *P. ostreatus* were placed at the center of each Petri dish containing the test media. The plates were sealed with masking tape, labeled, and incubated at 28°C for mycelial growth.

Measurement of mycelia growth: The vegetative growth of the mushroom was determined by measuring the diameter of the mycelium in the Petri dishes. The average mycelial extension and growth rate were calculated. The biomass, density and growth characteristics of the mushroom mycelia were also observed and determined. Statistical analysis was carried out using averages, means, and standard deviations.

Results

The study revealed that *P. ostreatus* and *M. inoderma* were capable of growing on all the tested culture media at varying degrees. All the media supported the growth of the mushrooms on culture plates. *Marasmiellus inoderma* fully colonized the Petri dish within an average of 3 to 4 days, while *Pleurotus ostreatus* required an average of 5 to 7 days to achieve complete colonization. After one day of inoculation, mycelial extension was highest for Czapek-Dox agar (19.00 ± 3.8 mm), followed by cornmeal agar (16.17 ± 2.3 mm) and yeast extract agar, while it was lowest for potato dextrose agar (13.67 ± 0.8 mm). However, after five days of inoculation, the highest mycelial growth was recorded for PDA (71.67 ± 3.3 mm) and cornmeal (71.33 ± 1.3 mm), followed by malt extract agar (67.50 ± 2.5 mm). This was not significantly different from Czapek-Dox agar (66.67 ± 1.2 mm) and wheat extract agar (66.50 ± 1.3 mm). Mycelial extension was least for rice extract agar after five days of inoculation (Table 1). *Pleurotus ostreatus* recorded the highest extension (27.83±1.6 mm) for Malt extract agar and the least for PDA (23.00±1.5 mm) after one day of inoculation. MEA and RCEA media gave the best mycelia extension of 84.00±1.7 mm and 84.00±1.7 mm, respectively, after 3 days of inoculation. Cornmeal recorded the least mycelia extension (519.83±4.3 mm) after 3 days of inoculation (Table 2).

The growth rate was observed to be faster for *Marasmiellus inoderma* for the media tested (MEA - 27.99±0.58 mm/day, Czapek-Dox - 27.59±0.38 mm/day) than for *Pleurotus ostreatus* (PDA -14.33±0.65 mm/day, Cornmeal – 14.27±0.25 mm/day). The growth rate was the least for Rice extract agar (23.61±0.9 mm/day) for M. *inoderma* and (11.43±1.12 mm/day for P. *ostreatus*, respectively. The result for the growth rate is shown in Figure 1.

The media type also influenced the mycelia biomass of the *M. inoderma* and *P. ostreatus* to varying degrees. The highest mycelial biomass was obtained for *M. inoderma* was recorded in MEA (962.00±0.05 mg), followed by wheat extract agar (892.00±54.67 mg), and least in Czapek-Dox media (507.67±14.64 mg). On the other hand, the highest mycelia biomass of *P. ostreatus* was recorded in PDA (770.67±18.77 mg), followed by MEA (742.33±28.88 mg). The least biomass for P. *ostreatus* was observed in Rice extract agar (621.35±18.82 mg). The result is shown in Table 3. The result revealed that the media influenced the mycelia density and cultural characteristics of the mycelia of the mushrooms under investigation. Tables 4 and 5 show the mycelia density and mycelia morphology of *M. inoderma* and *P. ostreatus*, respectively.

Table 1:.Effect of media on the mycelia extension (mm) of *Pleurotus ostreatus*.

	Daily mycelium extension (mm)				
Media	**1DAI**	**2DAI**	**3DAI**	**4DAI**	**5DAI**
PDA	*13.67 ± 0.8	25.50 ± 0.5	37.23 ± 2.8	58.67 ± 7.9	71.67 ± 3.3
CZPAK	19.00 ± 3.8	26.33 ± 2.8	37.33 ± 4.9	49.17 ± 4.2	66.67 ± 1.2
YEA	15.17 ± 1.0	25.67 ± 3.8	36.67 ± 2.8	49.83 ± 5.9	62.83 ± 2.9
MEA	14.50 ± 1.8	26.33 ± 1.0	43.83 ± 2.3	56.50 ± 2.5	67.50 ± 2.5
CRM	16.17 ± 2.3	25.83 ± 0.7	43.00 ± 2.6	59.17 ± 3.8	71.33 ± 1.3
WHT	14.33 ± 0.6	26.17 ± 0.8	39.17 ± 0.7	51.17 ± 2.8	66.50 ± 1.3
RCEA	15.45 ± 2.2	25.95 ± 1.7	39.12 ± 3.9	41.33 ± 2.3	57.17 ± 2.6

*Value= means ± standard deviation. DAI- Day after inoculation. PDA- Potato dextrose agar, CZPAK - Czapek-Dox, YEA- yeast extract agar, MEA- malt extract agar, CRM- cornmeal, WHT- wheat extract agar, RCEA- rice extract agar

Table 2: Effect of media on the mycelia extension (mm) of *Marasmiellus inoderma*

	Daily mycelium extension (mm)				
Media	**1DAI**	**2DAI**	**3DAI**	**4DAI**	**5DAI**
PDA	*23.00 ± 1.5	47.33 ± 3.2	73.67 ± 5.5		
CZPAK	24.84 ± 1.7	53.00 ± 0.8	82.67 ± 1.3		
YEA	27.16 ± 1.0	58.67 ± 0.5	82.00 ± 1.8		
MEA	27.83 ± 1.6	60.83 ± 5.2	84.00 ± 1.7		
CRM	23.83 ± 0.7	45.00 ± 0.8	59.83 ± 4.3		
WHT	19.67 ± 0.2	53.50 ± 138	81.83 ± 2.8		
RCEA	27.83 ± 1.6	60.83 ± 5.2	84.00 ± 1.7		

*Value= means ± standard deviation. DAI- Day after inoculation. PDA- Potato dextrose agar, CZPAK - Czapek-Dox, YEA- yeast extract agar, MEA- malt extract agar, CRM- cornmeal, WHT- wheat extract agar, RCEA- rice extract agar

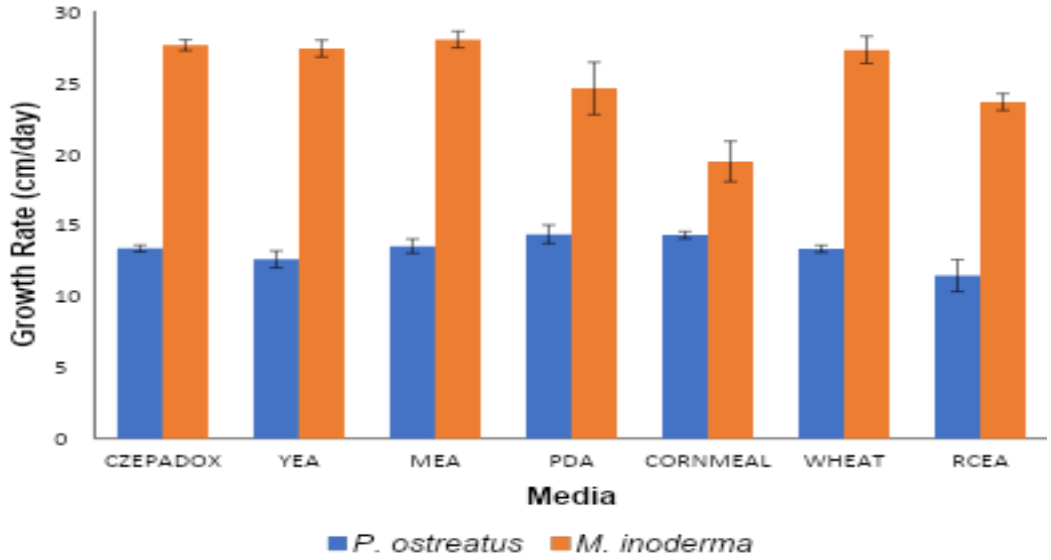


Figure 1: Effect of media on the growth rate (mm/day) of *Pleurotus ostreatus* and *Marasmiellus inoderma*
PDA- Potato dextrose agar, CZPAK - Czapek-Dox, YEA- yeast extract agar, MEA- malt extract agar, CRM- cornmeal, WHT- wheat extract agar, RCEA- rice extract agar

Table 3: Effect of media on the biomass of *Pleurotus ostreatus* and *Marasmiellus inoderma*

	Mushroom	
Medium	***M. inoderma***	***P. ostreatus***
PDA	602.00±30.00	770.67±18.77
CZAP	507.67±14.64	629.67±43.52
YEA	662.00±17.06	637.67±32.59
MEA	962.33±18.44	742.33±28.88
CRM	592.33±23.11	649.33±34.78
WHT	892.00±54.67	656.33±41.40
RCE	754.670±36.46	621.35±78.82

*Mean ± SD value of three replicates. PDA- Potato dextrose agar, CZPAK - Czapek-Dox, YEA- yeast extract agar, MEA- malt extract agar, CRM- cornmeal, WHT- wheat extract agar, RCEA- rice extract agar

Table 4: Effect of culture media on mycelia density and culture morphology of *Pleurotus ostreatus.*

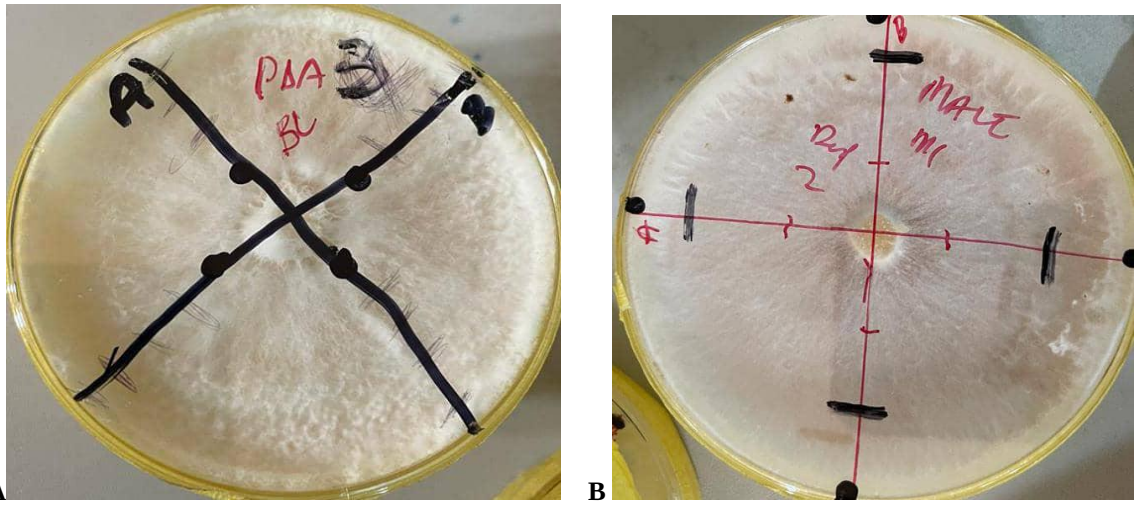
Media	**Density**	**Cultural Characteristics**
PDA	++++	Wooly/ enraised
CZPAK	+++	Appressed
YEA	++	Cottony
MEA	++++	Woolly/cottony
CRM	+	Appressed
WHT	+++	Wooly
RCEA	+++	Fluffy/woolly

PDA- Potato dextrose agar, CZPAK - Czapek-Dox, YEA- yeast extract agar, MEA- malt extract agar, CRM- cornmeal, WHT- wheat extract agar, RCEA- rice extract agar + = very thin, ++ = thin, +++ = dense, ++++ = very dense

Table 5: Effect of culture media on mycelia density and culture morphology of *Marasmiellus inoderma.*

Media	**Density**	**Cultural Characteristics**
PDA	++	Wooly
Czepak- dox	+	Appressed
YEA	++	Woolly/cottony
MEA	+++++	Woolly/eccentric
CRM	+	Appressed
WHT	+++	Wooly
RCEA	++++	Appressed

Key: + = very thin, ++ = thin, +++ = dense, ++++ = very dense



A **B**

Plate 1: Mycelial density of *P. ostreatus* (A) and *M. inoderma* (B) in culture plates

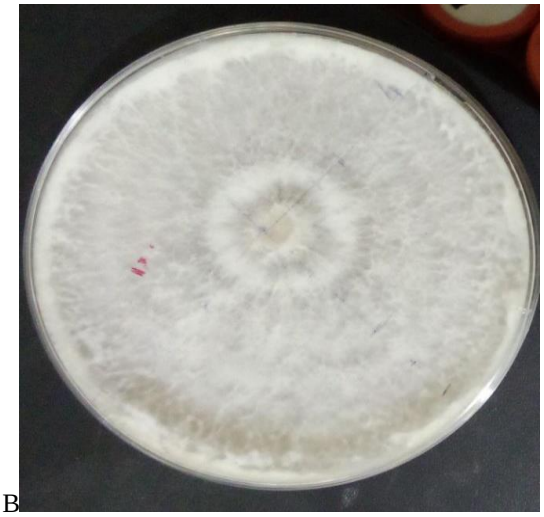
A  B 

Plate 2: Mycelial morphology of *P. ostreatus* (A) and *M. inoderma* (B) in culture plate

The results of this study confirm that culture media type significantly influence the vegetative growth of *Pleurotus ostreatus* and *Marasmiellus inoderma*. Mycelial extension, growth rate, biomass accumulation, and morphology varied significantly between the mushrooms species and across the tested media. *Marasmiellus inoderma* demonstrated more rapid colonization of the culture plates, achieving complete mycelial colonization within 3–4 days across the media, while *Pleurotus ostreatus* took 5–7 days to attain complete mycelial coloniztion.

Among the media tested, malt extract agar (MEA) supported the fastest mycelial extension rate for *M. inoderma* (27.99 ± 0.58 mm/day), followed closely by Czapek-Dox agar. For *P. ostreatus*, the highest growth rate was observed in potato dextrose agar (14.33 ± 0.65 mm/day), with cornmeal agar (14.27 ± 0.25 mm/day) also supporting substantial growth. Rice extract agar consistently recorded the lowest growth rate for both species. *M. inoderma* produced the highest mycelial biomass on MEA (962.33 ± 18.44 mg), while its lowest yield was recorded on Czapek-Dox agar. In contrast, *P. ostreatus* showed maximal biomass on PDA (770.67 ± 18.77 mg) and the lowest on rice extract agar (621.35 ± 78.82 mg).

The media composition also influenced the mycelial morphology and density. In *P. ostreatus*, the mycelia grew very densely, woolly to cottony on PDA and MEA but appressed, and thinner on cornmeal and Czapek-Dox agar. Likewise, *M. inoderma* produced extremely dense mycelium in MEA and rice extract agar, however, it produced sparse and pressed mycelia in Czapek-Dox and cornmeal agar.

Discussion

This differential growth dynamic is consistent with the enzymatic efficiency attributed to *M. inoderma*, including the production of phenoloxidases such as laccases, which facilitate rapid lignin degradation and nutrient assimilation (Farnet *et al*., 2002). The variation in biomass further supports these findings. These observations are consistent with prior studies that identified PDA and MEA as optimal media for *Pleurotus* spp. due to their rich carbohydrate and nitrogen content (Nguyen and Ranamukhaarachchi, 2020, Kartik *et al*., 2022, Pant *et al.*, 2020). The significant differences observed in the hyphal density and morphology implied that the media is a determining factor in fungi physiology, morphology and the production of fruiting bodies. The successful use of both synthetic and locally formulated media, such as rice and wheat extract agars, underscores the adaptability of these fungi and the feasibility of cost-effective mushroom cultivation using agricultural residues. Studies with comparative analysis of the effects of commercial culture media and natural media from the local wastes on fungal growth has been given special attention (Landingin *et al*., 2020, Arana-Gabriel *et al*., 2020, Dulay *et al.*, 2021). This is because it is not always possible to find the proper composition in synthetic and semi-synthetic media that would provide better growth than naturally originated nutrient medium (Rizal *et al*., 2016). Thus, the results become more obvious after comparison of mycelium growth activity obtained with engaging of differently originated nutrition media. Numerous reports describe the fungal growth on natural media based on local raw materials and found that the yield is higher or comparable to those obtained on commercial media. Investigations of medium content impact on the growth of fungal species such as *Pleurotus cystidiosus* and *P. ostreatus* (Hoa and Wang, 2015). The efficiency of natural media application therefore

contributes to a significant reduction in the cost of the final product and also focused on a waste-free production cycle.

Krupodorova *et al*. (2024) reports that natural media contain a sufficient amount of biologically active substances that are vital to fungal growth, and they can be used as mono-substrates. With this purpose, the natural media that is based on locally sourced predominantly plant-based or agricultural residues waste is a high-desirable and easy of approach.

These findings align with previous studies that demonstrated good mycelial growth of Mushroom species such as *Pleurotus* spp. and *Marasmiellus* species, on plant-based and waste-derived media (Ukoima *et al*., 2009, De Kesel *et al*., 2002). The presence of growth-promoting compounds, such as vitamins, amino acids, or trace elements, in these formulated substrates may have contributed to the observed enhancements in mycelial extension and density.

In addition, the high and prompt growth in *M. inoderma* is an indication that it can be utilized industrially or ecologically (in bioremediation and enzyme production). The high degree of reproducibility in media used in the cultivation of *P. ostreatus* and *M. inoderma*, is an indicator that they can be cultured on an industrial scale. These insights could enhance sustainable adoption of fungal biotechnology in food security and restoring the environment through agricultural activities.

In summary, this study demonstrates that both synthetic and cereal-based formulated media effectively support the vegetative growth of *Pleurotus ostreatus* and *Marasmiellus inoderma*, albeit with distinct species-specific responses across media types. The observed variation in mycelial extension, biomass accumulation, and morphological traits highlights the critical role of substrate composition in optimizing fungal development. These findings offer a valuable basis for future research into substrate formulation, spawn standardization, and the development of scalable protocols for sustainable mushroom cultivation and biotechnological applications.

Thus, the results become more obvious after comparison of mycelium growth activity obtained with engaging of differently originated nutrition media. Numerous reports describe the fungal growth on natural media based on local raw materials and found that the yield is higher or comparable to those obtained on commercial media (Aditya *et al*., 2021). Based on the findings of this study, it is recommended that locally formulated media such as rice, wheat, and corn-based agars be considered as effective and affordable alternatives to conventional synthetic media for mushroom cultivation. Further research should explore nutrient enhancement strategies to improve yield and biomass production, particularly for *Marasmiellus inoderma*, which showed rapid mycelial growth. The application of these findings at a commercial scale could support low-cost, sustainable mushroom production systems.

Natural media contain a sufficient amount of biologically active substances that are vital to fungal growth, and they can be used as mono-substrates. With this purpose, the natural media that is based on locally sourced predominantly plant-based or agricultural residues waste is a high-desirable and easy of approach. Various perennial and annual herbaceous plants have been tested as ingredients for the natural media (Aditya *et al*., 2022). Also, plants with a high potato-like starch content are promising candidates as a substrate for mushroom growth and include examples of Solanum tuberosum, Ipomoea batatas, Dioscorea spp. (Mshandete and Mgonja 2009, Hoa and Wang 2015, Landingin *et al*., 2020)). Media, tested by the investigators for fungi growth, differ by the composition of organic and inorganic compounds, and have been differently enriched with nutrients, such as carbohydrates, nitrogen sources, micro- and macro-elements, amino acids and vitamins.

Conclusion

This study has provided compelling evidence that the type of culture medium significantly affects the growth performance of *Pleurotus ostreatus* and *Marasmiellus inoderma*. *Marasmiellus inoderma* consistently demonstrated faster colonization and higher biomass yield, particularly in malt extract agar, highlighting its potential as a rapidly growing species of ecological and biotechnological relevance. The successful utilization of cereal-based formulations such as rice and wheat extract agar underscores the viability of low-cost, locally sourced alternatives to conventional synthetic media. This has important implications for sustainable mushroom cultivation, particularly in developing regions where commercial media may be inaccessible or economically not feasible. These findings contribute to the growing body of knowledge on fungal cultivation and support future research into spawn development, media enrichment, and scalable production systems for edible and medicinal mushrooms.

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