



Agaricales Production: A Systematic Review on its Representative Species' Cultivation Process and Substrate Influence

Riana D. Almodovar, Marybel R. Cornejo, Micah M. Mendoza,
and Joseph R. Naval

De La Salle University Integrated School, Biñan City, Laguna

Abstract: Mushroom cultivation has long been of economic importance, specifically in Asian nations where most mushrooms are grown and sold. The globally popular *Pleurotus ostreatus* and a mushroom with similar composition profiles to it, *Calocybe indica*, are gaining recognition among farmers in urban areas due to their low-cost production and ability to grow on diversified substrates. Assessing the cultivation of both *C. indica* and *P. ostreatus* with selected substrates has been unexplored by researchers, most especially systematic reviews that focus on the cultivation of mushrooms in the Philippines. This paper sought to find the effectiveness of wheat straw, paddy straw, and sugarcane bagasse substrates in increasing specific growth parameters of *C. indica* and *P. ostreatus*. A systematic review with a narrative synthesis approach was performed to determine the effects of the chosen substrates on growth and yield parameters. The study utilized the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) as a basis for reporting the results and the Cochrane risk-of-bias tool to appraise the studies included in the review critically. Results show that wheat straw had increased the rate of spawn run and pinhead formation of both mushrooms, while paddy straw obtained high yield parameters. Though there was a lack of substantiation of a leading substrate among the three, paddy and wheat straw are substrates that might have the potential in increasing the mushroom's yield and quality in the Philippines.

Key Words: mushroom cultivation; *Pleurotus ostreatus*; *Calocybe indica*; lignocellulosic substrates; systematic review

1. INTRODUCTION

Mushrooms are a group of macro-fungi unable to perform the process of photosynthesis. Therefore, they feed on the nutrients of organic matter by releasing enzymes that decompose their organic material. These fruiting bodies belong to the kingdom Fungi under Phylum Basidiomycota and Ascomycota. However, most edible and commercially cultivated fungi belong to Basidiomycota under the order of Agaricales (Rahi & Malik, 2016). One species under Agaricales would be the *Pleurotus ostreatus*, otherwise known as Oyster mushrooms. They are low in maintenance, easy to cultivate, and can tolerate and thrive in a wide range of temperatures and climatic conditions (Sbhatu et al., 2019; Bellettini et al., 2019). According to the National Horticulture Board (n.d.), *P. ostreatus* thrives in an environment with a temperature of 20°C to 30°C and humidity of 55% to 70%. They productively grow on various lignocellulosic wastes, making them simple to produce. One mushroom that is also capable of thriving over a selection of lignocellulosic wastes is *Calocybe indica*. This fungus is cultivated in South

India and other South Asian countries and is most suitable for tropical regions. This mushroom's cultivation is low-cost and can be grown throughout a year (Samonte, 2014). *C. indica* has a high fruit yield of 100% to 800% (Spowart, 2017) and is also less perishable than other fungi whose shelf life is only a week at room temperature. Though *C. indica* possesses properties similar to the *P. ostreatus*, no research that focused on assessing both *C. indica* and *P. ostreatus* with selected substrates as their focused subject was found.

Additionally, no systematic review focuses on the potent mushroom cultivation that concentrates on the Philippines. Thus, a systematic review is needed to analyze both mushrooms' substrate efficacy. This study aims to critically assess all relevant investigations related to Agaricales production in answering research questions that address the effectiveness of various lignocellulosic substrates in increasing specific growth and yield parameters of *C. indica* and *P. ostreatus*.

This review was confined to literature situated in Asia and had used wheat straw, paddy straw, and sugarcane bagasse as their substrates.



These agro-wastes are frequently used in a plethora of studies involving *P. ostreatus* and *C. indica* cultivation. While it was stated that this paper was limited to the three mentioned substrates, at least one of the three is required in a study due to the limited research conducted on the topic. Different growth and yield parameters were used to evaluate each substrate's influence, namely, spawn run, pinhead formation, total yield, and biological efficiency.

Spawn run and pinhead formation were the first two steps for mycelial growth that primarily focus on its substrate colonization duration. On the other hand, the total yield and biological efficiency exhibited the substrates' effect on the fruiting bodies' overall growth.

This study's findings will be beneficial to Filipino mushroom farmers, for a thorough analysis of the strategies and substrates in increasing mushroom production will be imparted to them. It offers an opportunity for milky mushrooms to be introduced to the Philippine mushroom industry. It also encourages rice and sugarcane farmers to invest in mushroom production since it is an environment-friendly alternative to managing their wastes. Additionally, future researchers may use these findings as evidence of the selected substrates' influence towards both mushrooms' growth parameters.

2. METHODOLOGY

2.1. Research Design

A systematic review was conducted to provide evidence of the substrate efficacy in the cultivation parameters of both *C. indica* and *P. ostreatus*. Studies were gathered and critically assessed in different criteria and biases to ensure the quality of the review's findings. A narrative synthesis, accompanied by graphical data presentations, was used in analyzing the results from the eligible literature.

2.2. Search Strategy

Due to physical limitations, purely electronic databases accessible through the University Library were used for gathering literature to review. These databases include the following: SciFinder, ScienceDirect, and AnimoSearch. Keywords were formed from the research questions combined with truncation symbols. Table 1 includes the keywords used with the respective database.

2.3. Inclusion and Exclusion Criteria

Table 1. Electronic databases utilized in the systematic review

Database	Search Strategy
SciFinder (15,367 results)	(<i>Calocybe indica</i> OR <i>Pleurotus ostreatus</i>) AND (~yield OR ~morphological properties OR ~Agronomic OR ~cultivation)OR(~paddy straw OR ~wheat straw OR~ sugarcane)OR (<i>Lyophyllaceae</i> OR <i>Pleurotoceae</i>)
ScienceDirect (35,387 results)	(<i>Calocybe indica</i> OR <i>Pleurotus ostreatus</i>) AND (yield OR morphological properties OR Agronomic OR cultivation)OR(paddy straw OR wheat straw OR sugarcane)OR (<i>Lyophyllaceae</i> OR <i>Pleurotoceae</i>) Year: 2013 to 2020
Animosearch (5,153 results)	(<i>Calocybe indica</i> OR <i>Pleurotus ostreatus</i>) AND (yield~ OR morphological* OR Agronomic~ OR cultivation*~)OR(paddy straw~ OR wheat straw~ OR sugarcane ~)OR (<i>Lyophyllaceae</i> * OR <i>Pleurotoceae</i> *)

To secure the eligible articles' quality, the researchers screened the studies through the inclusion and exclusion criteria. A study was included for the following reasons:

It included at least one of the three target substrates. It investigated the substrates' effects using the following parameters: spawn run, pinhead formation, total yield, and biological efficiency.

It focused on the cultivation of the *C. indica* or *P. ostreatus*.

It has an experimental research design with at least two replications of the experiment.

Its methodology was conducted in vitro and within an Asian country.

On the other hand, a study was excluded for the following reasons:

It addressed another concept aside from cultivation.

Its full-text cannot be accessed due to premium publication restrictions.

It was written in a language aside from English.

Its publication date exceeded seven years from the present time.

It was not a peer-reviewed article.

2.4. Study Selection

Researchers followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Flowchart for eligible articles' screening process. Titles and abstracts were first screened concerning the topic. Afterward, the inclusion and exclusion criteria were applied to the screened articles with their full text, followed by the critical assessment review. The remaining articles were considered eligible studies for the review. Data were then extracted, including the study design and characteristics, the substrate used, and the outcome parameters.

2.5. Risk of Bias Assessment

Risk of bias assessment was conducted following the Cochrane risk of bias tool to ensure validity and objectivity from the eligible articles. The following criteria were used in the assessment: (1) performance bias, (2) detection bias, (3) attrition bias, and (4) reporting bias. Assessments were rated as uncertain, high, or low. Results were presented in a table showing the included study and the degree of bias present based on the researchers' individual and group assessments.

3. RESULTS AND DISCUSSION

3.1 Study Selection

This systematic review included ten studies. A flow chart of the identification and inclusion of studies is presented in Figure 1.

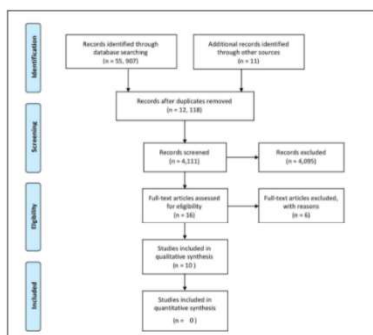


Figure 1. Flow chart of the study selection process in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement

3.2 Risk of Bias Assessment

The final ten journals chosen reached an overall judgment of Risk of Bias Assessment (RoB) of 75-100% of low risk and less than 25% rating of “some concerns,” most information across the journals were sufficiently low-risk bias. Despite some incomplete information, it is unlikely that this would affect the synthesized results.



Figure 2. Traffic Light plot and summary plot of the risk of bias assessment.

3.3 Spawn Run

Subbiah and Balan (2015) states that *C. indica*'s spawn run usually appears at 15 to 20 days while *P. ostreatus*' 2 to 3 weeks (Buah et al., 2010). In Table 2, it was observed that the reviewed journals followed these values with slightly larger ranges (*C. indica* - 15.00 to 23.20 days, *P. ostreatus* - 13.81 to 29 days).

Two substrates are tied as a preferred substrate for *C. indica*: paddy and wheat straw. Patel and Trivedi (2016) and Shrikhandia and Sumbali (2019) had paddy straw as their best substrate, while for Singh et al. (2019) and Vijaykumar et al. (2013), it was wheat straw. Similarly, wheat straw was also a suitable performing substrate for *P. ostreatus*, followed by sugarcane bagasse. Spawn run was significantly higher than other substrates in two studies (Abid et al., 2020; Yang et al., 2013).

Table 2. Mean spawn run (days) of *C. indica* across different studies

Substrate	Patel & Trivedi (2016)	Singh et al. (2019)	Vijaykumar et al. (2014)	Navathe et al. (2014)	Shrikhandia and Sumbali (2019)
Paddy straw	18.4	-	17.67	17	15.00
Wheat straw	20.2	18.44	15.67	-	15.93
Sugarcane bagasse	21.4	23.20	19.00	-	-

Table 3. Mean spawn run (days) of *P. ostreatus* across different studies

Substrate	Abid et al. (2020)	Zakil et al. (2020)	Zakil et al. (2019)	Sitaula et al. (2018)	Yang et al. (2013)
Paddy straw	25.83	-	-	18.25	-
Wheat straw	19.50	-	-	-	13.81
Sugarcane bagasse	-	29	26	20.00	-

3.4 Pinhead formation

The first growth milestone to a fruiting body, pinhead formation, signifies a mushroom's health (Ibrahim et al., 2017). For *C. indica*, it takes 10 to 28.67 days to form (Subbiah & Balan, 2015; Kumar et al., 2017). Similarly, *P. ostreatus* takes 16 to 27 days to develop pinheads (Buah et al., 2010).



Figure 3. Spawn Run (a), Pinhead Formation (b), and Pinhead Formation to Maturation (c) of *P. ostreatus* from Tesfay et al. (2020)



Figure 4. Spawn Run (a), Pinhead Formation (b), and Cropping Stage (c) of *P. ostreatus* from Kora (2020)

In Table 4, Shrikhandia and Sumbali (2019) and Navathe et al. (2014) had the paddy straw develop the fastest pinheads. Contrariwise, Singh et al. (2019) and Vijaykumar et al. (2014) had wheat straw as the best substrate. Note that in Singh et al. (2019), paddy straw was not able to cultivate mushrooms. *P. ostreatus*' best substrate for pinhead formation was identical to its spawn run: wheat straw. Occurring in two literature works (Yang et al., 2013; Abid et al., 2020), it was the fastest pinhead formation across journals. Following these values was sugarcane bagasse for *C. indica* and paddy straw for *P. ostreatus*. It is evident among both mushrooms that wheat straw is the most efficient for pinhead formation.

Table 4. Pinhead formation(days) of *C. indica* across different studies

Substrate	Patel & Trivedi (2016)	Singh et al. (2019)	Vijaykumar et al. (2014)	Navathe et al. (2014)	Shrikhandia and Sumbali (2019)
Paddy straw	405	-	1324	810.5	399.03
Wheat straw	298	320.04	1463	-	388.61
Sugarcane bagasse	255	221.8	515.7	-	-

Table 5. Pinhead formation(days) of *P. ostreatus* across different studies

Substrate	Patel & Trivedi (2016)	Singh et al. (2019)	Vijaykumar et al. (2014)	Navathe et al. (2014)	Shrikhandia and Sumbali (2019)
Paddy straw	18.66	-	-	21.75	-
Wheat straw	16.50	-	-	-	6.00
Sugarcane bagasse	-	30	28	23.25	-

3.5 Total yield

Despite the incomplete information, paddy straw was revealed to be a dominant substrate in terms of the average yield in the *C. indica*. Three out of five studies concluded this with wheat straw as the second-best substrate. Consequently, Vijaykumar et al. (2014) and Singh et al. (2019) had wheat as their preferred substrate with paddy straw following these values.

Studies on *P. ostreatus* show that wheat straw has a more significant influence than paddy straw in the mushroom's growth. This was supported by Abid et al. (2019) and Yang et al. (2013), with their total yield was highest on paddy straw. This was closely followed by paddy straw. Although sugarcane bagasse had a significantly high value in Sitaula et al.'s (2018) work, overall, it still had relatively lower values.

Table 6. Total yield (grams) of *C. indica* across different studies

Substrate	Patel & Trivedi (2016)	Singh et al. (2019)	Vijaykumar et al. (2014)	Navathe et al. (2014)	Shrikhandia and Sumbali (2019)
Paddy straw	405	-	1324	810.5	399.03
Wheat straw	298	320.04	1463	-	388.61
Sugarcane bagasse	255	221.8	515.7	-	-

Table 7. Total yield (grams) of *P. ostreatus* across different studies

Substrate	Abid et al. (2020)	Zakil et al. (2020)	Zakil et al. (2019)	Sitaula et al. (2018)	Yang et al. (2013)
Paddy straw	145.33	-	-	528.45	287.2
Wheat straw	160.5	-	-	-	287.43
Sugarcane bagasse	-	41.35	273.3	527.8	-

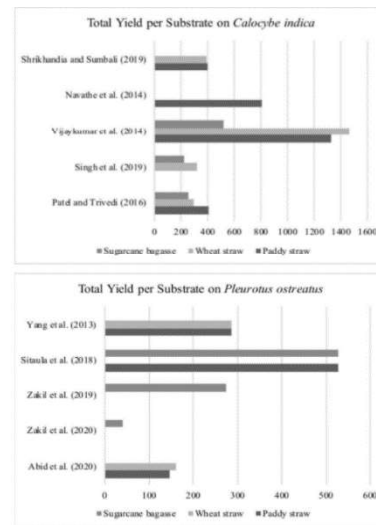


Figure 5. Total yield (grams) of *C. indica* (top) and *P. ostreatus* (bottom) across various studies

3.6 Biological Efficiency

Biological efficiency is the amount of yield per kilogram of substrate. Similar to the yield, paddy straw outperforms other substrates on the biological efficiency of *C. indica*. With a range of 77.6% to 134.86%, paddy straw is the best substrate in three out of four journals, excluding the studies that did not assess it. Having more divided views, among *P. ostreatus* studies, paddy has the overall highest biological efficiency in Sitaula et al. (2018) and Yang et al. (2013). In contrast, wheat straw was favored in Singh et al. (2019), followed by paddy straw. It can be said that paddy and wheat straw have a slightly similar performance.



Table 8. Biological Efficiency (Percentage) of *C. indica* across different studies

Substrate	Abid et al. (2020)	Zakil et al. (2020)	Zakil et al. (2019)	Sitaula et al. (2018)	Yang et al. (2013)
Paddy straw	24.38	-	-	78.33	78.73
Wheat straw	22.6	-	-	-	78.35
Sugarcane bagasse	-	44.95	68.33	71.91	-

Table 9. Biological Efficiency (Percentage) of *P. ostreatus* across different studies

Substrate	Patel & Trivedi (2016)	Singh et al. (2019)	Vijaykumar et al. (2014)	Navathe et al. (2014)	Shrikhandia and Sumbali (2019)
Paddy straw	134.86	-	132.4	81.05	79.8
Wheat straw	85.07	64	146.3	-	77.6
Sugarcane bagasse	85.02	44.36	51.57	-	-

in early primordial formation.

Additionally, Amin et al. (2010) expressed that cellulose-rich substrates are also responsible for higher yield. With a relatively higher cellulose content than sugarcane bagasse, wheat and paddy straw initiated high yield performance in *P. ostreatus* and *C. indica*. Lastly, paddy straw has the second-highest C/N ratio and highest cellulose content. It outperformed the other two substrates in yield parameters but had equal performance in growth parameters to wheat straw. This could be due to the abundance of both the C/N ratio and cellulose content.

Table 10. Composition of substrates (Ahmed et al., 2011; Bakker et al. 2013; Ferreira et al., 2016; Lindley et al., 2017; Sakdaramarong et al., 2012; Sharma et al., 2014)

Substrate	C/N ratio	Cellulose (%)	Hemicellulose
Paddy straw	90:1	37.5	30
Wheat straw	80:1	33.5	25
Sugarcane bagasse	100:1	33	24

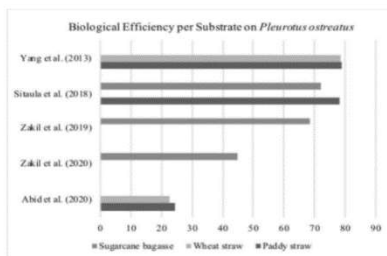
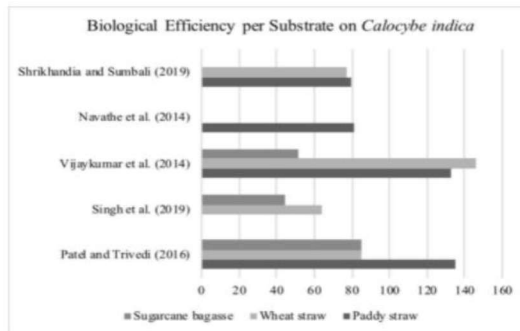


Figure 6. Biological Efficiency (Percentage) of *C. indica* (top) and *P. ostreatus* (bottom) across different studies

3.7 Effect of the composition of substrates

It is speculated that the composition of the substrates affected the growth and yield of the mushroom. Ahmed et al. (2009) and Elahe et al. (2016) reported that the right amount of hemicellulose, cellulose, lignin, and the carbon-nitrogen (C/N) ratio is accountable for quick spawn run. Hoa et al. (2015) also stated that an adequate amount of C/N ratio is desired for mycelium growth. However, a lower ratio is better for the creation of primordia that develop into fruiting bodies. Wheat straw has the lowest C/N ratio out of all the chosen substrates as seen in Table 10. Hence, the contents of wheat straw fulfill the nutritional demand of both mushrooms, which results

4. CONCLUSIONS

The studies reviewed sufficiently provided detailed information that indicated wheat straw seemed to outdo the other substrates on spawn run and pinhead formation for both *C. indica* and *P. ostreatus*. Simultaneously, paddy straw excelled on total yield and biological efficiency for both mushrooms. To some extent, this suggests that both wheat straw and paddy straw as substrates may be acceptable for use in specific parameters by farmers and producers, depending on the targeted parameter that needs concentration. No evidence supported which substrate outdid all other substrates alone, considering both parameters and mushrooms. Instead, the results from studies situated in Asian countries similar to the Philippines' climate suggested that the application of wheat straw and paddy straw substrates is worth investing in to attain more efficient production. Results provided limited coverage of the influences the substrates have from various set-ups and environmental effects. This emitted implications on the validity of mushroom cultivation evaluations.

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