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A preliminary study on culture media preparation from vegetable waste

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ABSTRACT

A culture medium is an environment that provides essential nutrients, moisture, and physical support to sustain the growth of microorganisms in a laboratory setting. While Potato Dextrose Broth (PDB) has been a valuable tool for fungal cultivation in liquid media, exploring alternative methods could lead to more sustainable, cost-effective, and customizable options for laboratory research and biotechnological applications. This study aimed to find out the potential of utilizing various mixtures of vegetable wastes such as cabbage, onion, carrot, beans, beetroot, and drumstick in different ratios to cultivate selected fungi, *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp. and *Trichoderma* sp. under laboratory conditions. Three different mixtures were used: BCC (beetroot, carrot, cabbage), OBD (onion, beans, drumstick) and BCO (beans, carrot, onion). Most of the fungi showed greater growth on alternative media compared to Potato Dextrose Broth (PDB). The results indicated that the BCC medium which was prepared in 1:1:2, 1:1:1 and 1:2:1 ratios, exhibited higher growth for *Aspergillus* sp., *Fusarium* sp. and *Mucor* sp. respectively. The OBD medium highly induced the growth of *Rhizopus* sp. and *Trichoderma* sp., which were prepared in 1:1:1 and 1:1:2 ratios, respectively. Lastly, higher development of *Penicillium* sp. was observed in the BCO medium, which was mixed in a 2:1:1 ratio. Thus, vegetable waste serves as a useful source for fungal cultivation and it can replace the expensive commercial culture media.

Keywords: Alternative media, Potato Dextrose Broth, Vegetable waste, Cost-effective, Spore count.

1. INTRODUCTION

Microorganisms, the unseen giants of our world, wield immense power and influence despite their diminutive size and are ubiquitous. They encompass a vast array of life forms, ranging from bacteria and archaea to fungi, protists, and viruses. Despite their small size, they exhibit remarkable diversity in shape, structure and function. Among these, fungi play an imperative role in day-to-day life. Fungi are a diverse group of organisms, and they're categorized as yeast, molds, and mushrooms based on their cellular nature, where yeast are unicellular and molds and mushrooms are multicellular. They also play a pivotal role in shaping ecosystems and impacting human lives in various ways. From the beneficial role of fungi in medicine and agriculture to their harmful effects on human health and infrastructure, the dual nature of fungi underscores the complexity of their interactions with the environment [1]. Fungal molds are involved in the production of antibiotics, industrial enzymes, organic acids, organic solvents, biopesticides, extracellular proteins, and other metabolites [2]. Beyond these, they can act as biocontrol agents against some pathogenic organisms and some polymers and also be involved in the biotreatment of wastewater ingredients such as metals, inorganic nutrients, and organic compounds [3]. Due to these reasons, fungi are cultivated under laboratory conditions. To cultivate fungi, they need an appropriate environment that should provide all

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the necessary nutrients and growth conditions, which is called culture media.

It can be in liquid, semi-solid, or solid form. Culture media typically contain sources of carbon, nitrogen, vitamins, minerals, and other growth factors necessary for the organisms to thrive, but their composition varies depending on the type of microorganism or cells being cultured and the specific experimental requirements. Specifically, carbon concentration and the C:N ratio highly affect fungal growth and sporulation, as well as being strain-dependent. Therefore, it is essential to consider the complexity of nutritional requirements to improve fungal production [4]. Lots of media are commercially available to grow fungi, like Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Corn Meal Agar (CMA), and Rose Bengal Agar (RBA), which are high in cost [5].

While these media provide convenience and consistency, they also introduce some drawbacks that researchers and industries need to consider when using them for fungal cultivation. Since they are very expensive, the cost of ready-made media can become a big restriction, particularly when huge culturing is required for research or production purposes, especially in laboratories with limited financial requirements or for large-scale industrial purposes. Other than these, commercial fungal culture media typically have a limited shelf life, especially once opened, due to the risk of microbial contamination and degradation of nutrients. Thus, recent researchers have been involved in finding an alternative way to save money by using readily available, inexpensive ingredients without compromising fungal growth and productivity [6].

On the other hand, waste management is a global problem, especially in developing countries. Increasing waste and properly managing the waste is becoming a serious threat. Approximately 40% - 60% of waste is produced by households in the urban environment. These wastes can be divided into two types: dry waste and wet waste [8]. Mainly, wet waste contains organic waste like fruits and vegetable waste, which includes peels, seeds, pomace, stems, pods, etc. Such wastes are rich in carbohydrates and other nutrients, which can be easily utilized by microorganisms [9].

Solid waste management presents many challenges, from collection and transportation to treatment and disposal. One of the main challenges is the large amount of waste generated, especially in urban areas. Insufficient infrastructure and resources increase this issue, leading to poor waste collection and disposal systems. Additionally, improper waste disposal practices, such as open dumping and burning, have created overflowing landfills that contribute to environmental degradation in terms of groundwater pollution and contribute to global warming by releasing methane and carbon dioxide [5].

Since these kinds of vegetable wastes contain complex carbohydrates such as cellulose, hemicellulose, and pectin, which can serve as the primary energy source for fungi, they provide the necessary carbon for growth and metabolism. Therefore, it can be used to make culture medium, and this natural source of nutrients can support robust fungal proliferation without the need for synthetic additives, promoting a more environmentally friendly approach to culture media.

There has been lot of research done on using vegetable waste as an alternative culture medium. Sayali *et al*., [8], used pomegranate peels, sweet lime and orange pomace (leftover after extraction of juices), peapods, and spinach stems as a mixture to cultivate *Aspergillus* sp., *Penicillium* sp., *Candida albicans,* and *Saccharomyces cerevisiae,* where fungal growth was faster on kitchen waste agar than commercially available Sabouraud's media. All standard fungal cultures showed growth after 24 hours on kitchen waste agar while Sabouraud's media took 48hours to show growth, at room temperature. Another experiment was performed to test the ability of starch-containing tubers like sweet potato verities (purple and white) and yam and cocoyam verities (edible and non-edible) as culture media to replace potatoes in a general-purpose culture medium. *Aspergillus niger* and *Aspergillus carbonarius* were used as test organisms. Among these, purple potatoes highly induced the

mycelial growth of these fungi when compared to other formulations because of their high carbohydrate content [10].

One of the researchers used *Canavalia ensiformis* (Linn) (jack beans) as a carbon and nitrogen source to grow six species of fungi: *Aspergillus flavus*, *Aspergillus niger, Meria coniospora, Mucor sp., Neurospora crassa, and Rhizopus oryzae*where, *Aspergillus flavus* showed the highest biomass of 1.70 g in the media formulated with *Canavalia ensiformis* as the carbon source [15]. Chanda *et al.* [6] used onion peel, garlic peel, and corn peel (GCO) as a mixture to grow *Penicillium chrysogenum, Aspergillus niger,* and *Trichoderma viridae.* All of the three fungal cultures grew better and faster in the GCO medium compared to regularly used media. Another study was carried out to find alternative media for the growth of *Aspergillus* sp. and *Trichoderma* sp. using drumstick, potato peel, cauliflower stalk, and fenugreek stem. Alternative media strongly supported the growth of these fungi when compared to PDA [7]. Rosemay *et al.* [14] formulated a mycological medium utilizing varying concentration of tomato juice extract as a substitute for Potato Dextrose Agar to cultivate various fungi including *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Rhizopus stolonifer, Penicillium digitanum* and *Fusarium oxyporum*. The medium demonstrated considerable efficiency in promoting fungal growth, indicating its suitability for application in mycological research.

Each fungus needs specific carbon and nitrogen requirements for its growth as an energy source. Since, such agro-industrial and kitchen wastes contain a large amount of nutrients, vitamins, minerals, and bioactive compounds, they can be used to produce an alternative medium that can support the growth of microorganisms under laboratory conditions with minimal contamination as it does not meet the needs of every microbe [11]. The present study was aimed to find a cost-effective medium using kitchen wastes, especially vegetable waste, to grow six different fungi namely *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., and *Trichoderma* sp.

2. METHODOLOGY

2.1 Collection and preparation of samples

Kitchen wastes such as cabbage, onion, carrot, beans, beetroot, and drumstick were collected from home kitchen and cut into small pieces. They were left to dry under the shade to remove the moisture until they reached a constant weight. Dried samples were ground to a fine powder using a mechanical grinder which was then stored in airtight, clean and dry containers for further usage.

2.2 Preparation and maintenance of fresh fungal cultures

The test organisms such as *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., and *Trichoderma* sp. were obtained from the culture collection section of the Department of Botany, University of Jaffna and sub-cultured on Potato Dextrose Agar (PDA) for 2-3 days at room temperature [7]. Stock cultures were also maintained on agar slants.

2.3 Preparation of fungal spore suspension

Spores were obtained from a three days old culture, and spore suspension was prepared in a saline solution (0.85% NaCl) at an initial concentration of 1×10^4 spores/ml. The suspension was then incubated for two hours at room temperature to stimulate the spores' metabolic and growth processes (Modified from [1]).

2.4 Preparation of vegetable peel waste media and Potato Dextrose Broth (PDB)

Three distinct media were formulated by combining three vegetable waste: BCC (beetroot, carrot, cabbage), OBD (onion, beans, drumstick) and BCO (beans, carrot, onion). The mixtures were prepared in four different ratios: R_1(1:1:1), R_2(2:1:1), R_3(1:2:1) and R_4(1:1:2). A total of 2 g of dried, powdered vegetable waste mixture was added in 50mL of distilled water and they were sterilized through autoclaving. All sets were prepared in triplicate. PDB media was prepared as a control for this study.

2.5 Inoculation of fungal suspension

1 ml of the above prepared spore suspension was inoculated using micropipette to each prepared vegetable waste media and Potato Dextrose Broth (PDB) and they were incubated at room temperature for 3 to 5 days [1].

2.6 Qualitative and Quantitative analysis of fungal growth

Fungal growth on vegetable waste media and PDB was visually monitored and recorded as qualitative results from 3rd to 5th day after inoculation. The spore concentration in each media was determined by counting spores using a haemocytometer on the 3rd, 4th, and 5th days, and the data was represented in a graph. Additionally, the dry weight of the fungi was measured after the 5th day by filtering the fungal culture through Whatman No. 1 filter paper.

2.7 Statistical analysis

All the experiments were carried out in triplicate, and the mean values were used to plot the graphical representations. Statistical analyses were performed using Minitab 20. The data were analyzed in a one-way ANOVA. Tukey's pairwise comparison test was used to determine the significance difference at $p< 0.05$.

3. RESULTS AND DISCUSSION

Six different fungi (*Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., and *Trichoderma* sp.) were used and they were maintained on Potato Dextrose Agar (PDA) throughout this study. The growth of each fungus on vegetable waste media and Potato Dextrose Broth (PDB) was initially examined through visual identification and the results are shown in Table 1.

Table 1. Qualitative growth analysis of fungus on vegetable waste media and PDB

Fungus	BCC	OBD	BCO	PDB
***Aspergillus* sp.**	++++	+++	+++	+++++
***Fusarium* sp.**	++	+	+	+
***Mucor* sp.**	++++++	+++++	++++++	++++++
***Penicillium* sp.**	+	++	++++	++
***Rhizopus* sp.**	+++++	++++++	+++++	++++
***Trichoderma* sp.**	+++	++++	++	+++

Same amount of (+) signs shared by two different fungi will not indicate the same amount of growth.

According to visual identification, *Mucor* sp., *Rhizopus* sp., and *Aspergillus* sp. are the most dominant fungi that grew well almost in all vegetable waste media when compared to *Trichoderma* sp., *Fusarium* sp. and *Penicillium* sp. Despite this, nearly all of the fungi's development was significantly facilitated by vegetable waste media than PDB. Qualitative results may vary among people because it can be influenced by individual perspectives and experience. As such, a quantitative analysis was done. The following outcomes were obtained when spore concentration and mycelial dry weight were considered as quantitative factors.

3.1 Effect of Different BCC Media Formulations on Fungal Spore Concentration and Mycelial Biomass Production

3.1.1 Spore concentration analysis

Mucor sp. achieved the highest sporulation on R_3 medium with 6.06×10^6 spores/mL spore concentration indicating the affinity for the nutrient distribution in this formulation. On PDB, however, the *Mucor* sp. produced 4.01×10^6 spores/mL of spore concentration indicating that the BCC medium has a greater potential for the development of *Mucor* sp. (Figure 3).

Aspergillus sp. also exhibited the highest sporulation (5.85×10^6 spores/mL) next to *Mucor* sp. on R_4 media. In contrast, its sporulation was comparatively lower on R_2 formulation with 4.01×10^6 spores/mL spore concentration, reveal that variations in nutrient ratios significantly affect spore production. R_3 was found to be the least effective formulation for the sporulation of *Penicillium* sp. which exhibited the lowest spore production among all fungi with 1.67×10^6 spores/mL spore concentration (Figure 3). However, a significant increase was observed on R_2 media, where it is produced nearly 4.43×10^6 spores/mL spore concentration. *Trichoderma* sp. exhibited moderate spore concentrations, where its highest value was observed in the R_2 formulation (3.93×10^6 spores/mL) and lowest in the R_4 formulation (1.96×10^6 spores/mL).

3.1.2 Mycelial dry weight analysis

Mycelial dry weight data provide the vegetative growth of fungi across different media formulations. Highest amount of mycelia (0.9335 g) was produced by *Mucor* sp. on R_4 medium after five days of incubation. Whereas, *Penicillium* sp. showed the least vegetative growth among other fungi, with the highest dry weight in R_4 formulation (0.2446 g) and the lowest growth in R_3 formulation with 0.0084 g of mycelia, showing its sensitivity to different ratios (Figure 4).

For most of the fungi, higher mycelial dry weight did not always correspond to higher spore concentration. For instance, *Aspergillus* sp. displayed the highest dry weight (0.7337 g) in R_1 medium but achieved maximum sporulation (5.85×10^6 spores/mL) in R_4 medium. Similarly, *Trichoderma* sp. showed a higher performance in R_2 in terms of sporulation (3.93×10^6 spores/mL) and maximum dry weight (0.5753 g) in R_1. Whereas PDB resulted in lower dry weight and spore concentration across all fungi and highlighting that the custom media formulation is crucial for applications requiring large biomass and suitability for producing fungal spores for agricultural or industrial applications. Preferences varied among fungal species, reflecting their unique metabolic requirements.

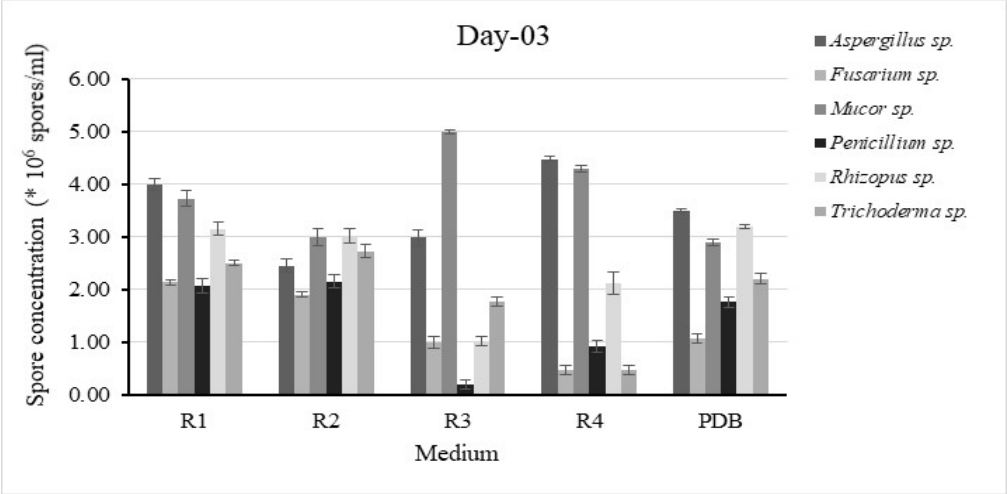

Figure 1. Spore concentration ($\times10^6$ spores/ml) of fungal isolates grown in different BCC media formulations after 3 days of incubation. R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1:2.

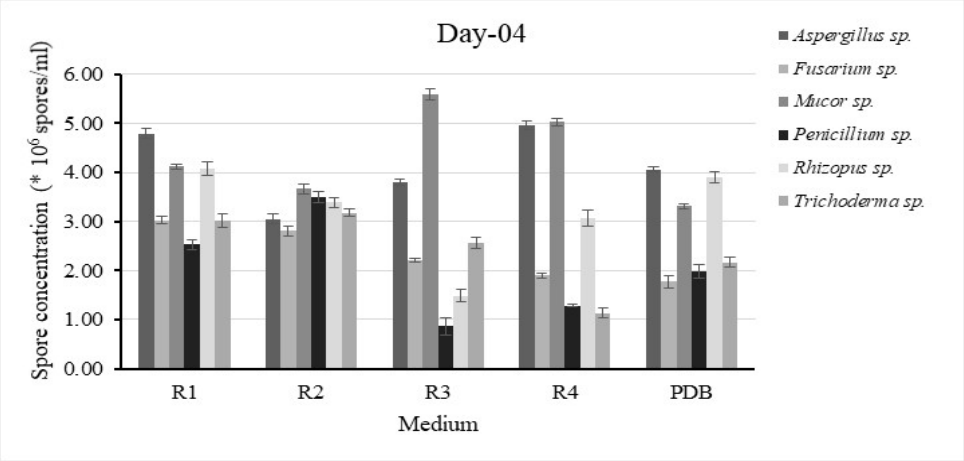

Figure 2. Spore concentration (×10⁶ spores/ml) of fungal isolates grown in different BCC media formulations after 4 days of incubation R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1

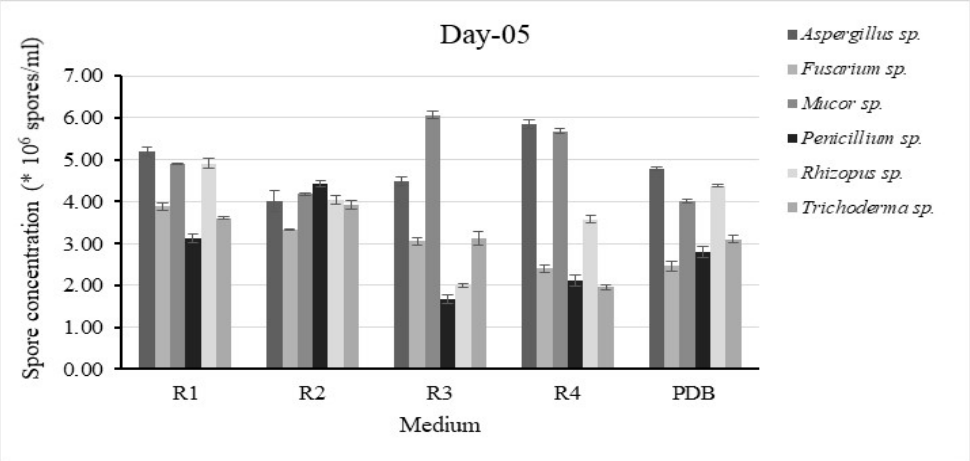

Figure 3. Spore concentration (×10⁶ spores/ml) of fungal isolates grown in different BCC media formulations after 5 days of incubation. R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1:2.

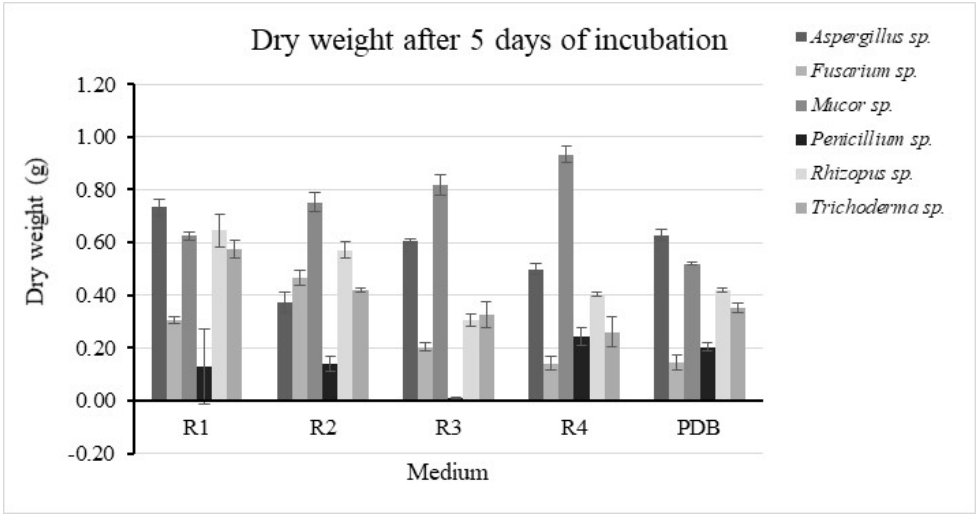

Figure 4. Mycelial dry weight of fungal isolates grown in different BCC media formulations after 5 days of incubation. R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1:2.

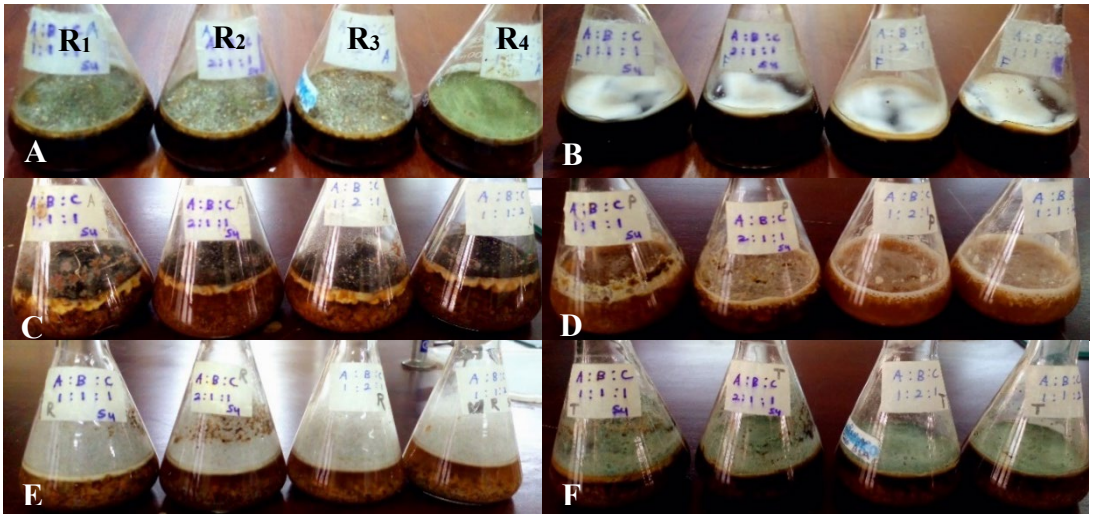

Figure 5. Fungal cultures across various BCC media formulation after five days of incubation: A- *Aspergillus* sp., B- *Fusarium* sp., C- *Mucor* sp., D- *Penicillium* sp., E- *Rhizopus* sp., F- *Trichoderma* sp., R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1:2.

3.2 Effect of Different OBD Media Formulations on Fungal Spore Concentration and Mycelial Biomass Production

3.2.1 Spore concentration analysis

Rhizopus sp. produced the highest spore concentration across all the media formulations, especially in R_1 with 6.10×10^6 spores/mL. Additionally, its spore production was also high in R_3 (5.53×10^6 spores/mL) and R_2 (4.87×10^6 spores/mL), showing that it can adapt in multiple nutrient conditions. *Aspergillus* sp. also has significant spore production across most of the media formulations, with the highest spore production (5.92×10^6 spores/mL) in R_2. The R_4 (5.06×10^6 spores/mL) and R_1 (4.35×10^6 spores/mL) media also supported relatively good spore production, while R_3 medium significantly restricted its sporulation (2.81×10^6 spores/mL) (Figure 8). The results reveal that the *Aspergillus* sp. and *Rhizopus* sp. showed steady growth across different formulations showing their adaptability to diverse nutrient environment. In contrast, *Penicillium* sp. and *Fusarium* sp. exhibited lower spore production compared to other fungi. Where, the higher spore production of *Penicillium* sp. was observed in R_1 with 3.78×10^6 spores/mL. But its spore production decreased in other formulations especially in R_4 media (1.70×10^6 spores/mL). Similarly, *Fusarium* sp. also showed limited sporulation in the same media with 0.42×10^6 spores/mL of spore concentration indicating more dependence on specific formulation.

3.2.2 Mycelial dry weight analysis

In the analysis of mycelial dry weight, *Mucor* sp. produced the highest biomass production (1.0186 g) in R_2 after five days of incubation. Significant dry weights of *Mucor* sp. were also recorded in R_3 (0.7991 g) and R_1 (0.6751 g). This indicates its adaptability to the diverse formulation of OBD. Meanwhile, the lowest mycelial production was recorded in *Fusarium* sp. (0.0077 g) in R_4. Similarly, *Penicillium* sp. produced highest mycelial weight (0.3004 g) in R_1, but showed significant reduction in other formulations (Figure 9).

These results showed that fungi such as *Mucor* sp. and *Rhizopus* sp. have significant adaptability across all OBD media formulations while *Fusarium* sp. and *Penicillium* sp. have greater sensitivity to nutrient variations in terms of sporulation and mycelial growth in different OBD media formulations.

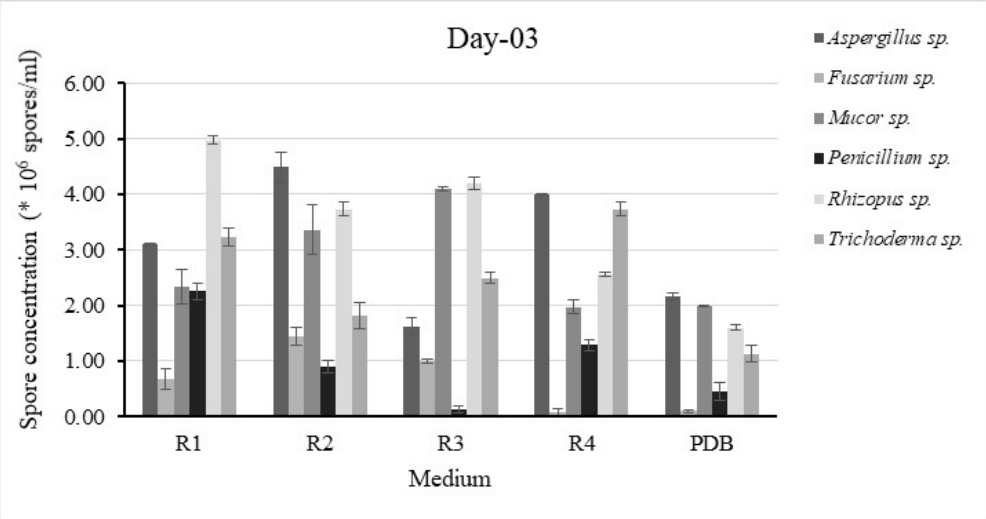

Figure 6. Spore concentration (×10⁶ spores/ml) of fungal isolates grown in different OBD media formulations after 3 days of incubation. R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1:2.

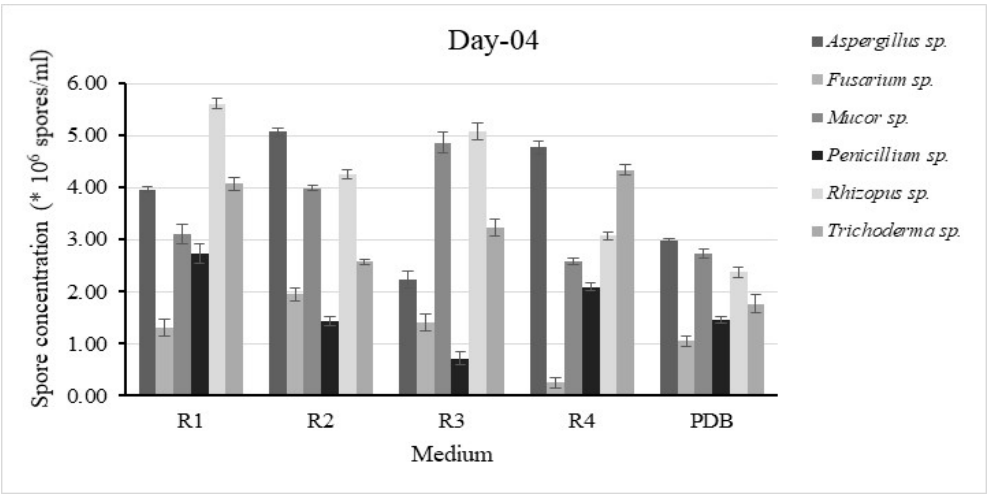

Figure 7. Spore concentration (×10⁶ spores/ml) of fungal isolates grown in different OBD media formulations after 4 days of incubation. R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1:2

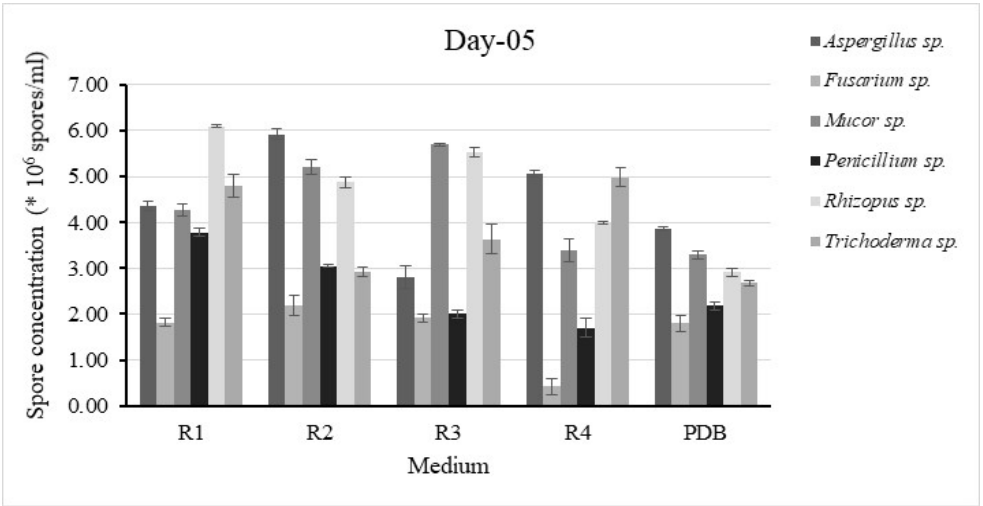

Figure 8. Spore concentration (×10⁶ spores/ml) of fungal isolates grown in different OBD media formulations after 5 days of incubation. R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1:2.

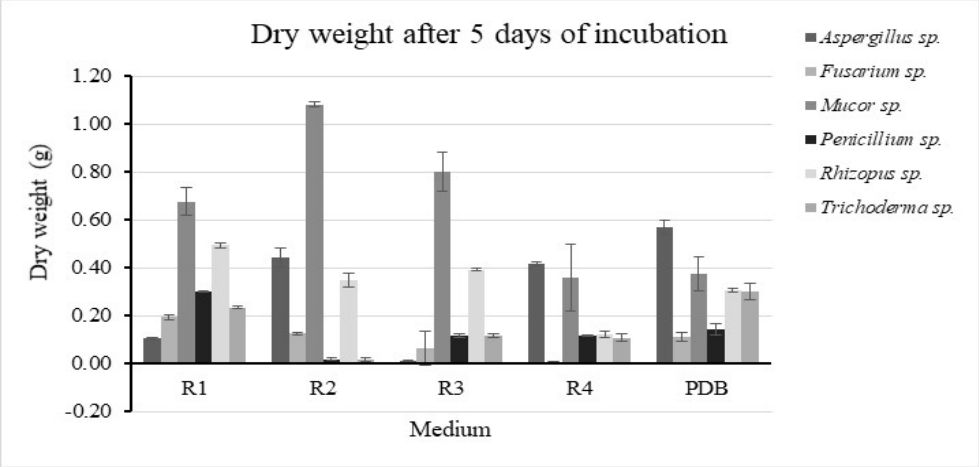

Figure 9. Mycelial dry weight of fungal isolates grown in different OBD media formulations after 5 days of incubation. R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1:2

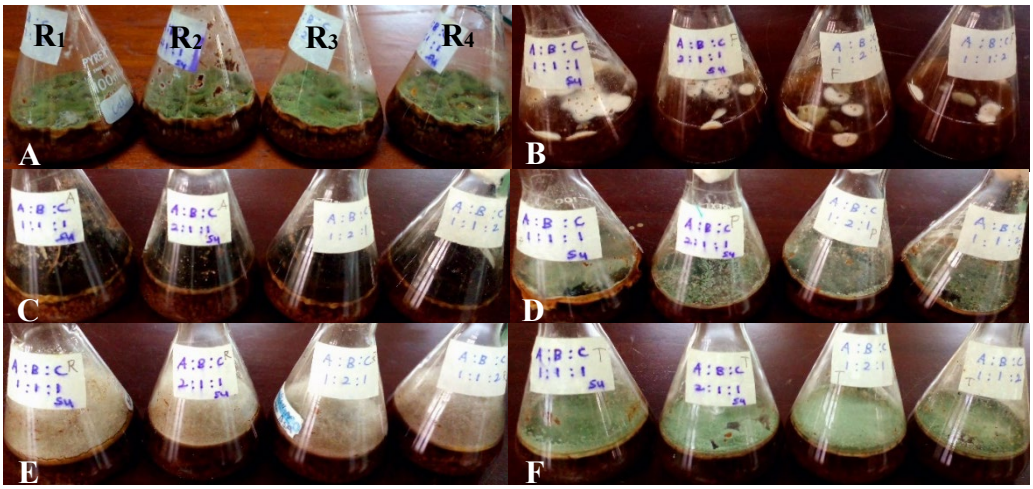

Figure 10. Fungal cultures across various OBD media formulation after five days of incubation: A- *Aspergillus* sp., B- *Fusarium* sp., C- *Mucor* sp., D- *Penicillium* sp., E- *Rhizopus* sp., F- *Trichoderma* sp., R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1:2.

3.3 Effect of Different BCO Media Formulations on Fungal Spore Concentration and Mycelial Biomass Production

3.3.1 Spore concentration analysis

In terms of spore concentration *Aspergillus* sp. showed the highest sporulation (6.35×10^6 spores/mL) in R_3, while *Rhizopus* sp. had the highest spore count (5.12×10^6 spores/mL) in R_1. *Mucor* sp. also exhibited the higher spore production R_1 (5.03×10^6 spores/mL) suggesting that these media formulations provide the necessary nutrients for the optimal spore production of these fungi (Figure 13). In contrast, *Fusarium* sp. and *Penicillium* sp. displayed comparatively less spore production. The highest spore concentration of *Fusarium* sp. (3.19×10^6 spores/mL) and *Penicillium* sp. (4.19×10^6 spores/mL) was observed in R_1 and R_2 respectively but produced significantly fewer spores in other formulations especially, *Fusarium* sp. produced just 1.10×10^6 spores/mL spores in R_4. However, their overall spore production was lower compared to other fungi (Figure 13).

3.3.2 Mycelial dry weight analysis

When considering mycelial dry weight *Mucor* sp. achieved the highest biomass production in R_2 with 0.5810 g of mycelia after five days of

incubation. *Rhizopus* sp. also showed highest growth next to *Mucor* sp. in R_1 with 0.5068 g of mycelia. Similarly, *Aspergillus* sp. showed moderate growth across different formulations. Especially, they produced 0.4422 g of mycelia in R_4, though R_3 media also support for the significant mycelial production (0.3758 g), highlighting how fungi allocate resources nutrients for mycelial production. On the other hand, *Fusarium* sp. and *Penicillium* sp. showed lower mycelial production almost in all formulations. Where, particularly *Penicillium* sp. showing very low biomass production in R_4 with 0.0153 g of mycelia (Figure 14). *Fusarium* sp. also produced lower amount of mycelia across all media formulation but slightly better growth in R_1 with 0.1921 g of mycelia.

The results showed that the *Aspergillus* sp., *Mucor* sp. and *Rhizopus* sp. are more adaptable fungi, which can proliferate across a broader range of nutrient condition in different BCO media formulations. In contrast, *Fusarium* sp. and *Penicillium* sp. are more selective and require more specific media formulation for optimal growth.

The comparison of the growth of each fungus in response to spore concentration and dry weight of mycelium is explained in detail in Table 2 and Table 3

Table 2. Comparison of spore concentration of each fungus in different media.

Fungi	Spore concentration
Aspergillus sp.	BCC > BCO > PDB > OBD
Fusarium sp.	BCC > BCO > OBD > PDB
Mucor sp.	BCC > OBD > BCO > PDB
Penicillium sp.	BCO > OBD > BCC > PDB
Rhizopus sp.	OBD > BCO > BCC > PDB
Trichoderma sp.	OBD > BCO > PDB > BCC

BCC medium seems to have a higher potential for spore production when compared to the other two mixtures, while OBD medium have relatively lower ability for sporulation. According to the chart, sporulation of *Aspergillus* sp., *Fusarium* sp., and *Mucor* sp. were high on BCC medium especially in R_4, R_1, and R_3 formulations respectively. Meanwhile, spore production of *Rhizopus* sp. and *Trichoderma* sp. was high on R_1 and R_4 formulations of OBD medium. Similarly, BCO was the most effective medium for the spore production of *Penicillium* sp., especially R_1 formulation. However, almost all the fungi showed significantly higher spore production compare to PDB.

Table 3. Comparison of mycelial dry weight of each fungi in different media.

Fungi	Dry weight
Aspergillus sp.	BCC > PDB > BCO > OBD
Fusarium sp.	BCC > BCO > OBD > PDB
Mucor sp.	OBD > BCO > BCC > PDB
Penicillium sp.	OBD > BCO > BCC > PDB
Rhizopus sp.	BCC > BCO > OBD > PDB
Trichoderma sp.	BCC > PDB > BCO > OBD

BCC medium was found to have a higher potential to induce vegetative growth of fungus, whereas OBD medium have significantly lower potential for the mycelial growth in most of the fungi that were used in this study. In particular, we saw that the mycelial dry weight of *Aspergillus* sp., *Rhizopus* sp., and *Trichoderma* sp. was high on R_1 formulation of BCC medium and *Fusarium* sp. in R_2formulation, while the mycelial dry weight of *Mucor* sp. and *Penicillium* sp. was high on R_2 and R_1 formulations OBD medium respectively. At the same time, *Fusarium* sp. and *Rhizopus* sp. produce a minimal amount of mycelia on OBD medium, especially in R_4formulation and *Aspergillus* sp. and *Trichoderma* sp. showed less mycelial dry weight in a R_3 and a R_2formulation of OBD medium.

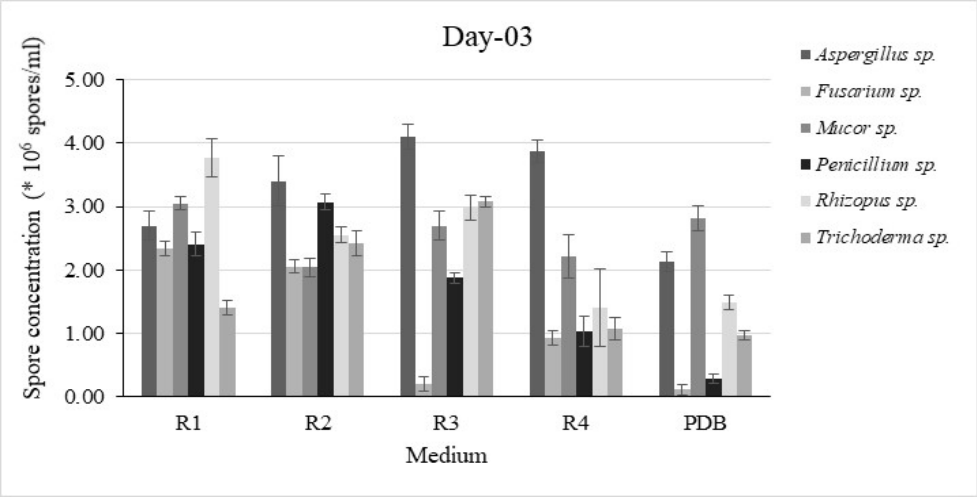

Figure 11. Spore concentration (×10⁶ spores/ml) of fungal isolates grown in different BCO media formulations after 3 days of incubation. R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1:2.

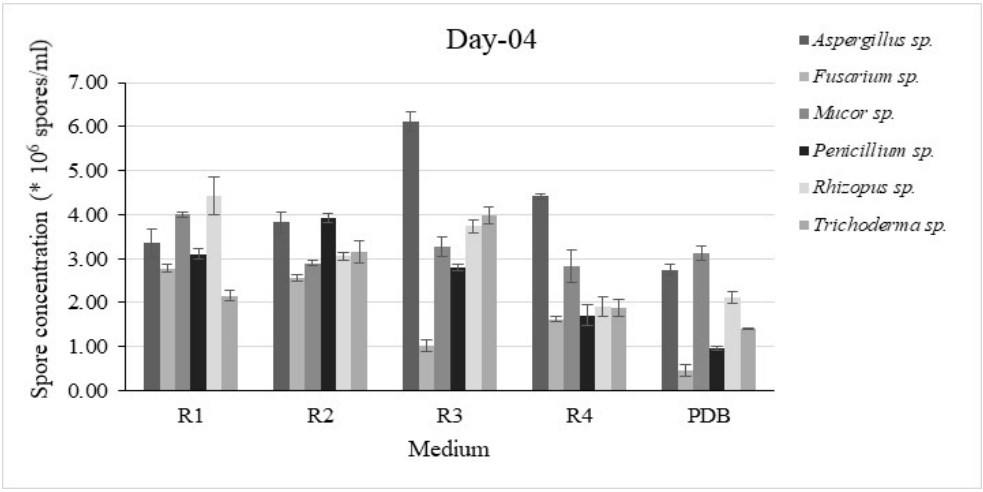

Figure 12. Spore concentration (×10⁶ spores/ml) of fungal isolates grown in different BCO media formulations after 4 days of incubation. R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1:2.

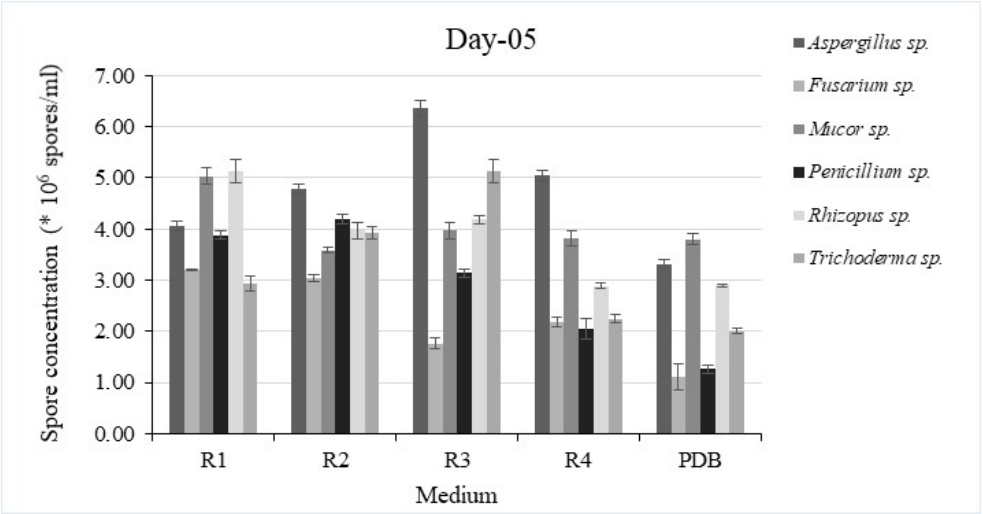

Figure 13. Spore concentration (×10⁶ spores/ml) of fungal isolates grown in different BCO media formulations after 5 days of incubation. R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1:2.

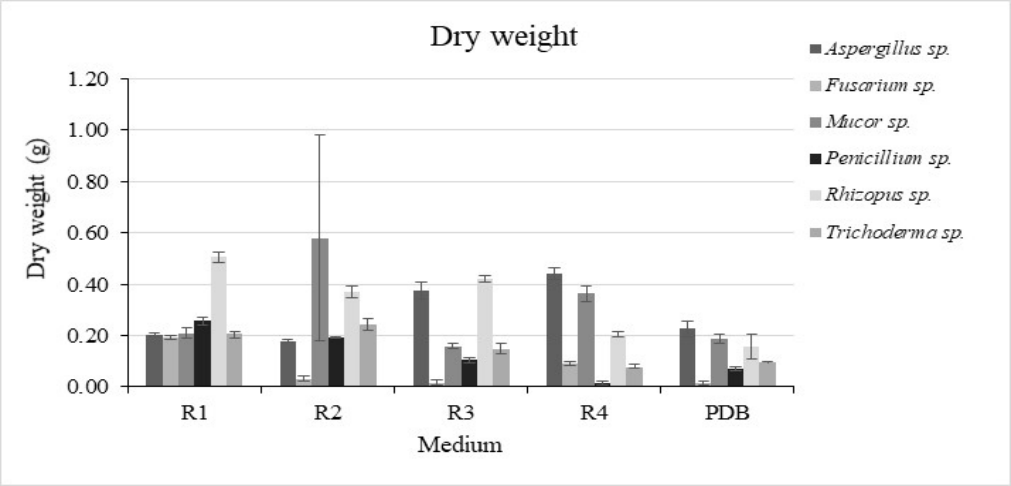

Figure 14. Mycelial dry weight of fungal isolates grown in different BCO media formulations after 5 days of incubation. R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1:2

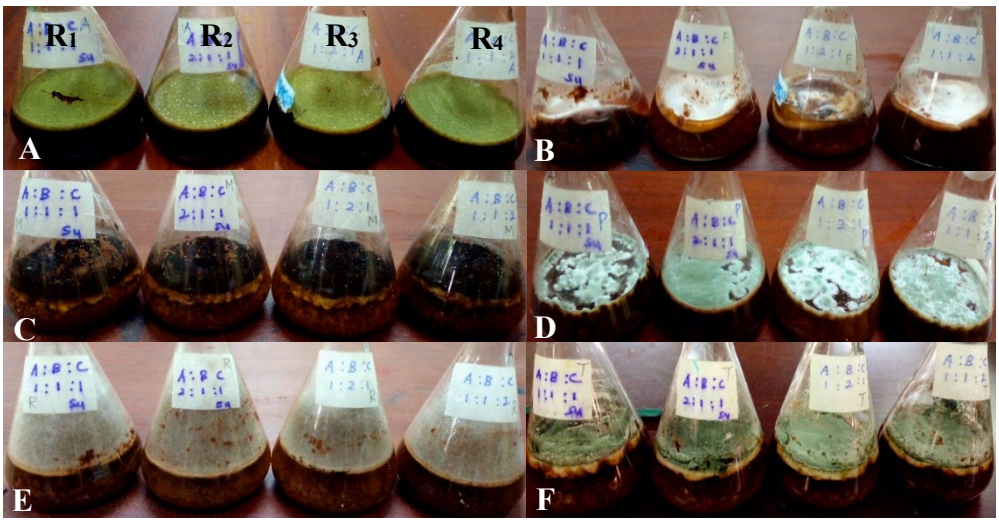

Figure 15. Fungal cultures across various BCO media formulation after five days of incubation: A- *Aspergillus* sp., B- *Fusarium* sp., C- *Mucor* sp., D- *Penicillium* sp., E- *Rhizopus* sp., F- *Trichoderma* sp., R_1- 1:1:1, R_2- 2:1:1, R_3- 1:2:1, R_4- 1:1:2.

All the vegetable waste was dried in the shade at the initial stages. This will assist in halting the degradation of metabolites and microbial fermentation. Shade drying will help to reduce the chemical reactions that happen under the influence of ultraviolet rays. Moreover, it's helpful to protect against discolorations and the loss of vital vitamins and minerals from vegetable wastes [12]. Fungal spores were inoculated in a 0.85% saline solution to prepare spore suspension, which helps to balance the osmotic pressure of fungi [5].

Sporulation and mycelial growth were examined in this study. Spores are the primary reproductive component, which involves the propagation of fungi. Fungi produce various types of spores for sexual (ascospores, basidiospores, zygospores and oospores) and asexual (sporangiospores, conidiospores, chlamydospores, etc.) reproduction. Mycelium is the vegetative portion of fungi, which may also be involved in reproduction. Mycelia of certain filamentous fungi may get into fragments and develop into new individuals. Some modified

mycelium, like sclerotia and rhizomorphs, also act as reproductive structures that are able to survive adverse environmental conditions [13]. Here, asexual spores were considered for counting, since *Rhizopus* sp. and *Mucor* sp. produced sporangiospores and *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., and *Trichoderma* sp. produced conidiospores.

Sporulation is influenced by environmental conditions and nutritional factors like carbon sources, nitrogen sources and microelements. Every fungus has specific carbon and nitrogen requirements for sporulation as well as mycelial growth. Protein content can supply a good source of nitrogen, while carbon is provided by carbohydrates. At the same time, fungal metabolism can be stimulated by the mineral content of the medium. In addition, water is also required for metabolic processes, especially extracellular digestion. Likely all the media were prepared using water, thus, the moisture content of the sample is negligible [9]. Therefore, different fungi show different growth rate on the same medium in response to spore concentration and mycelial dry weight.

4. CONCLUSION

This study has revealed that vegetable waste contains adequate requirements for the growth of fungi, and it can serve as a cost-effective method for fungal cultivation. All the tested fungi showed higher growth in the alternative medium compared to Potato Dextrose Broth (PDB). The percentage of each vegetable waste can affect the growth of fungi when a medium is made as a mixture of vegetable waste because of the variations in nutrient content. BCC medium was found to have a higher ability for the proliferation of fungi, while OBD medium exhibited minimal potential for fungal growth. However, the growth of all the selected fungi on OBD was high compared to PDB. Therefore, we can conclude that vegetable waste can serve as a good source for the preparation of fungal culture media, and it could also be one of the most effective way to minimize the environmental pollution.

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