

The Viability of Tender Coconut Water as a Medium for Fungal Cultures

S. Shayanthavi^{1a*}, R. Kapilan^{2b} and I. Wickramasinghe^{3a}

Abstract: Potato Dextrose Agar (PDA) is one of the most widely used media for fungal cultivation due to its nutrient-rich composition and consistent support for fungal growth. However, its reliance on potato extracts and relatively high cost have driven interest in identifying more sustainable and affordable alternatives in regions with limited access to commercial media. In this study, tender coconut water (TCW), the sterile, nutrient-rich liquid endosperm of immature coconuts, was explored as a potential supplementary or supportive medium for fungal cultivation. TCW-based agar media were formulated and compared with conventional PDA by assessing fungal growth based on colony diameter and dry biomass production. The results demonstrated that 100% TCW supported the highest fungal growth across all tested concentrations. To further enhance its effectiveness, the TCW medium was optimized using the Taguchi method by varying agar concentration, dextrose supplementation, and pH. The optimized conditions varied among fungal species. *Mucor* sp. and *Penicillium chrysogenum* showed maximum biomass in media containing 1% agar, no added dextrose, and a pH of 5.6. *Fusarium oxysporum* grew best in media with 1.5% agar, 1% dextrose, and a pH of 4.6, while *Aspergillus flavus* achieved optimal growth at 1% agar, 1% dextrose, and a pH of 6.6. Although TCW supported greater colony expansion, the fungal dry weight remained higher in PDA, showing superior biomass production. Nevertheless, tender coconut water demonstrated its potential as a natural medium for fungal growth. While TCW may not completely replace PDA, it shows promise as a cost-effective supportive medium, particularly in tropical regions or where resources are limited.

Keywords: Colony diameter, dry weight, fungal growth, tender coconut water.

1. Introduction

A good microbial growth medium requires a balanced supply of macro- and micronutrients. Primary growth-promoting elements include carbon and nitrogen, while trace elements such as sulfur, phosphorus, and various vitamins serve as vital micronutrients. In a laboratory setting, fungal growth is strongly influenced by the nutrients available in the media, which are essential for their growth, viability and reproduction (Laurie et al., 2015). Microbial culture media are crucial for the enrichment, isolation, and cultivation of microorganisms. Numerous fungal growth media are derived from staple crops that are vital food sources for humans, including corn, malt, oats, and potatoes. A prominent example is Potato Dextrose Agar (PDA), which consists of potato extracts, dextrose, and agar, making it one of the most widely used growth media for fungal cultivation. However, powdered PDA is relatively expensive and relies on potatoes, potentially reducing their availability in the market (Hasanin & Hashem, 2020). The rising cost of microbiological media has driven the development of novel formulations that prioritize both efficiency and affordability (Sawiphak et al., 2021).

In response to these limitations, recent research has increasingly focused on utilizing agro-industrial residues and naturally derived plant-based fluids as viable alternatives to conventional synthetic media. Examples of such substrates include tomato juice (Aliyu et

al., 2017), coconut-malt extract (Evangelista et al., 2021), soybean (Malik et al., 2022), Papaya fruit (Oktari et al., 2023), and watermelon peel extract (Hasanin & Hashem, 2020). These materials are typically rich in carbohydrates, minerals, and bioactive compounds that can support microbial growth.

Tender coconut water (TCW), the sterile liquid endosperm of immature coconuts (*Cocos nucifera* L.), presents a promising alternative medium due to its unique nutritional composition and availability in tropical regions. The use of coconut water in microbial culture was first documented in the 1960s, when it was found to support the growth of *Acetobacter xylinum*, the bacterium responsible for producing bacterial cellulose in "Nata de coco" fermentation (Alaban, 1962). Coconut water is naturally sterile within the unopened fruit, reducing the risk of initial contamination when used in microbial cultures (Tuyekar et al., 2021). Coconut water contains simple sugars (glucose, fructose, and sucrose), amino acids, vitamins (B-complex and vitamin C), minerals (potassium, magnesium, calcium, and phosphorus), and a small amount of protein. Its slightly acidic pH and presence of essential growth factors support the growth of various microorganisms (Rethinam & Krishnakumar, 2022). These nutrients provide carbon and micronutrient sources essential for fungal growth. Furthermore, TCW is a natural source of amino acids, vitamins, and plant hormones such as cytokinins, which may enhance fungal development and metabolic activity. Its high water content creates a favourable environment for fungal growth, while its chemical composition may offer comparable nutrient support to synthetic media in some cases (Burns et al.,

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2020). In developing countries, the necessity of creating culture media from locally sourced materials has become increasingly significant. Traditional media are often either difficult to access or prohibitively expensive, posing challenges for researchers and laboratories.

The high costs associated with these conventional media are largely due to economic instability and the limited purchasing power of local currencies. By utilizing locally available resources, researchers can reduce costs and improve the sustainability of their work (Wongjirattithi & Yottakot, 2017).

Due to its rich nutritional profile and availability, coconut water has already been applied in plant tissue culture as an organic supplement (Srinivasan et al., 2021). However, its potential as a stand-alone medium for fungal cultivation has not been thoroughly investigated. Therefore, this study aimed to evaluate the viability of tender coconut water as a natural, alternative growth medium for cultivating selected fungi such as *Fusarium oxysporum*, *Aspergillus flavus*, *Mucor* sp., and *Penicillium chrysogenum*. By comparing the growth of these fungi on TCW-agar with that on PDA, the study contributes to understanding whether TCW can be employed either as a supportive or supplementary medium in fungal culture practices.

The selected fungi represent a diverse group of species with both agricultural and environmental significance. *Penicillium* species play a significant role in promoting plant growth and enhancing soil health. They solubilize phosphorus by releasing organic acids, which lower the pH and convert insoluble forms into plant-available nutrients. As effective plant growth-promoting fungi (PGPF), they suppress various plant pathogens primarily through antibiotic production and by stimulating plant defense mechanisms. Their ability to adapt to diverse environments and tolerate abiotic stresses enhances their effectiveness in agricultural systems. *Penicillium chrysogenum* has shown potential in bioremediation, particularly in cadmium-contaminated soils, indicating the environmental value of this genus (Srinivasan et al., 2020). *Fusarium* species are agriculturally important, particularly as phytopathogens responsible for wilts and root rots in many crops, while also being producers of harmful mycotoxins. *Mucor*, a fast-growing zygomycete, is commonly found in soil and decaying organic matter and is often used as a model organism in fungal biology due to its rapid growth and ease of cultivation (Kour et al., 2019). *Aspergillus* is a widely distributed soil fungus commonly found in agricultural fields. Species like *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus fumigatus* are frequently isolated from crop rhizospheres. They play a major role in soil ecosystems and are influenced by environmental and agronomic factors. Some species produce harmful mycotoxins such as aflatoxins and ochratoxins, posing risks to food safety, while others are useful in industrial applications like enzyme production (Abdel-Azeem et al., 2019).

2. Material and Methods

Tender Coconut Water (TCW) Collection

Tender coconut water from coconuts aged 5 to 7 months was obtained from local farmers in Jaffna district. The coconuts were cleaned with chlorinated water, and the endosperm was obtained by removing the mesocarp and endocarp. The extracted water was filtered using Whatman filter paper to remove any debris (Shayanthavi et al., 2024).

Preparation of Growth Media

The test fungi were cultivated in Potato Dextrose Agar as the control medium and in Tender Coconut Water Agar (TCW-agar) as the test medium. TCW-agar was prepared by adding 1.5 g agar to 100 ml of fresh tender coconut water. A digital pH meter was used to measure the pH of the media to ensure proper acidity levels. Subsequently, the media underwent sterilization through autoclaving at 121°C for 15 to 20 minutes to eliminate microbial contamination. A control medium was prepared by mixing 3.9 grams of potato dextrose agar with 100 milliliters of distilled water, followed by autoclaving for sterilization. Once sterilized, the media were allowed to cool. It is crucial to ensure the medium has cooled adequately to prevent damage to the agar surface and to maintain aseptic conditions during the pouring process. Then, it was poured into petri dishes with a diameter of 9 cm under aseptic conditions to create a solid agar surface suitable for fungal growth (Sawiphak et al., 2021).

Comparison of The Physicochemical Characteristics of PDA and TCW-Agar

After media preparation, the physicochemical properties such as pH, colour and clarity were compared between potato dextrose agar (PDA) and tender coconut water agar (TCW-agar) (Adesemoye & Adedire, 2005).

Test Organisms and Inoculation of Media

The test fungi such as *Fusarium oxysporum*, *Penicillium chrysogenum*, *Aspergillus flavus*, and *Mucor* sp., were sourced from the Department of Botany, Faculty of Science, University of Jaffna. These fungi were initially cultured on Potato Dextrose Agar (PDA) media and incubated for 3 to 5 days. Afterward, they were preserved in PDA slants at 4°C for future use. The effectiveness of the chosen formulated media was assessed by cultivating them with the test fungi. The test fungi were inoculated onto fresh Potato Dextrose Agar (PDA) plates using a sterilized cork borer and incubated at 25°C for four days. The formulated media were inoculated using a sterile cork borer, which was sterilized with alcohol and flame, to create holes along the edge of the fungal starter cultures. Mycelial agar plugs were removed with a sterilized wire needle and placed in the center of the media. The plates were then incubated at room temperature. This process was repeated for each of the four test fungi from the pure cultures, inoculating them onto TCW-agar, water agar, and commercial PDA using the same method. Water agar is used as a negative control because it lacks the nutrients needed for

substantial fungal growth, containing only water and agar (Sawiphak et al., 2021).

Optimization of Media

One-factor-at-a-time (OFAT) and Taguchi methods were used for optimization of culture conditions (Azin et al., 2007). Initially, the concentration of tender coconut water (TCW) was optimized using the OFAT approach. Different concentrations of TCW, ranging from 20% to 100% (v/v), were prepared and tested to identify the most effective concentration for the culture medium. Subsequently, the Taguchi method was used to optimize other factors such as agar concentration, dextrose concentration and pH (as outlined in Table 1). The Taguchi method is a systematic experimental design that efficiently evaluates the effects of multiple factors while minimizing the number of experimental trials required. In this study, a Taguchi L9 orthogonal array implemented in Minitab 17 software was used to evaluate the effects of agar concentration, dextrose concentration, and pH on fungal growth. Nine experimental runs were conducted according to the L9 orthogonal array (Hasanin & Hashem, 2020).

Table 1. New medium formulation through the Taguchi method

Run	Agar concentration w/v (%)	Dextrose concentration w/v (%)	pH
F1	0.5	0	4.6
F2	0.5	1	5.6
F3	0.5	2	6.6
F4	1.0	0	5.6
F5	1.0	1	6.6
F6	1.0	2	4.6
F7	1.5	0	6.6
F8	1.5	1	4.6
F9	1.5	2	5.6

Determination of Fungal Growth

The growth of selected fungi on PDA and TCW-agar media was evaluated by measuring the colony diameter at room temperature (28 ± 2 °C). This involved inoculating sterile petri dishes (9 cm in diameter, containing 25 ml of media per plate) with 5 mm diameter mycelia agar plugs obtained from the edges of 4-day-old actively growing colonies of fungi grown on the respective formulated medium. Then diameter of growth was measured on the 3rd, 4th, 5th, and 6th days and growth of fungi in TCW-agar was compared with PDA (Marikar, 2009).

Estimation of Biomass Weight

The agar plates were subjected to 30 minutes of heating in a steamer on the 6th day of fungal growth to facilitate agar melting. Subsequently, the undamaged mycelium was rinsed once with distilled water and then transferred onto dried and pre-weighed filter paper. This filter paper with the mycelium was then placed in an aluminum dish and dried in an oven at 80°C for a duration of 18 hours. After cooling to room temperature inside a desiccator, the filter papers with the mycelium were weighed, and the dry weight was determined by calculating the difference in weight (Koley & Mahapatra, 2015).

Statistics

All tests were conducted in triplicate to ensure the reliability of the results. Statistical analyses were performed using Minitab 17 software. The results are expressed as mean $\pm$ standard deviation (SD). A significance level of $p \leq 0.05$ was utilized to determine statistical significance. Differences among groups were assessed using Analysis of Variance (ANOVA).

3. Results and Discussion

Physicochemical Properties of TCW-agar Media and PDA

The physicochemical characteristics of the TCW-agar media indicated the lowest pH value, whereas PDA exhibited the highest pH level. TCW-agar media appeared pale yellow, contrasting with the yellow colour of PDA. The absence of visible particles in the coconut water media or PDA suggests they are homogeneous and free from any discernible solid contaminants. Both TCW-agar media and PDA transformed into clear liquids after autoclaving.

Effect of TCW Concentration on Growth of Fungi

Table 2 presents the mean diameter of fungal growth on various concentrations of TCW-agar media and PDA at specific time points. The development of growth suggests that the fungi were able to adapt and proliferate in the respective environments provided by the TCW-agar, indicating their ability to utilize the available nutrients and conditions for their growth. As per the results from Table 2, it was consistently evident that the largest colony diameters were observed in 100% coconut water across all concentrations from 20% to 100% on the 3rd, 4th, 5th, and 6th day. By the 6th day, the colony diameters of all selected fungi on 100% TCW were measured as follows: *Fusarium oxysporum* (7.48 cm), *Aspergillus flavus* (8.17 cm), *Mucor* sp. (8.45 cm), and *Penicillium chrysogenum* (3.58 cm). However, when grown on PDA, the colony diameters were lower: *Fusarium oxysporum* (6.15 cm), *Aspergillus flavus* (7.25 cm), *Mucor* sp. (5.05 cm), and *Penicillium chrysogenum* (2.65 cm). This indicates a significant difference in colony diameter between PDA and 100% TCW-agar media, with PDA consistently yielding smaller colonies.

Table 2. The mean growth diameter of fungi on different concentrations of TCW-agar media and PDA on the 3rd, 4th, 5th and 6th day

		Diameter (cm)					
		20 %	40%	60%	80%	100%	PDA
<i>Fusarium oxysporum</i>	3D	3.23 ± 0.16 ^c	3.42 ± 0.15 ^{bc}	3.57 ± 0.09 ^b	3.53 ± 0.18 ^{bc}	3.92 ± 0.19 ^a	3.35 ± 0.27 ^{bc}
	4D	4.35 ± 0.27 ^{cd}	4.67 ± 0.14 ^{bc}	4.67 ± 0.12 ^{bc}	4.97 ± 0.19 ^{ab}	5.23 ± 0.23 ^a	4.13 ± 0.16 ^d
	5D	5.42 ± 0.23 ^d	5.87 ± 0.23 ^c	6.03 ± 0.28 ^{bc}	6.30 ± 0.14 ^{ab}	6.62 ± 0.23 ^a	5.13 ± 0.18 ^d
	6D	6.37 ± 0.12 ^{cd}	6.55 ± 0.10 ^c	6.57 ± 0.23 ^c	7.08 ± 0.12 ^b	7.48 ± 0.16 ^a	6.15 ± 0.10 ^d
<i>Aspergillus flavus</i>	3D	3.13 ± 0.27 ^d	3.55 ± 0.24 ^c	4.37 ± 0.23 ^b	4.92 ± 0.22 ^a	5.23 ± 0.16 ^a	4.05 ± 0.19 ^b
	4D	4.07 ± 0.19 ^e	4.70 ± 0.22 ^d	5.92 ± 0.15 ^b	6.32 ± 0.37 ^b	6.98 ± 0.21 ^a	5.18 ± 0.32 ^c
	5D	4.97 ± 0.31 ^e	6.48 ± 0.31 ^{cd}	7.12 ± 0.33 ^{bc}	7.30 ± 0.63 ^b	8.00 ± 0.21 ^a	6.32 ± 0.29 ^d
	6D	6.11 ± 0.15 ^d	7.60 ± 0.30 ^{bc}	7.72 ± 0.15 ^b	7.93 ± 0.23 ^{ab}	8.17 ± 0.22 ^a	7.25 ± 0.29 ^c
<i>Mucor sp.</i>	3D	2.92 ± 0.28 ^d	3.63 ± 0.60 ^{bc}	3.97 ± 0.22 ^b	4.80 ± 0.47 ^a	5.18 ± 0.47 ^a	3.23 ± 0.20 ^{cd}
	4D	3.48 ± 0.19 ^e	4.32 ± 0.28 ^d	5.47 ± 0.15 ^c	6.42 ± 0.19 ^b	6.87 ± 0.18 ^a	3.75 ± 0.10 ^e
	5D	4.32 ± 0.25 ^e	5.57 ± 0.18 ^d	6.85 ± 0.24 ^c	7.78 ± 0.34 ^b	8.20 ± 0.14 ^a	4.32 ± 0.15 ^e
	6D	5.67 ± 0.37 ^d	6.58 ± 0.28 ^c	7.93 ± 0.12 ^b	8.30 ± 0.14 ^{ab}	8.45 ± 0.15 ^a	5.05 ± 0.10 ^e
<i>Penicillium chrysogenum</i>	3D	1.38 ± 0.12 ^b	1.65 ± 0.19 ^{ab}	1.73 ± 0.18 ^{ab}	1.87 ± 0.20 ^a	1.93 ± 0.27 ^a	1.72 ± 0.29 ^{ab}
	4D	1.92 ± 0.12 ^b	2.08 ± 0.23 ^{ab}	2.17 ± 0.31 ^{ab}	2.50 ± 0.38 ^a	2.58 ± 0.36 ^a	2.18 ± 0.34 ^{ab}
	5D	2.42 ± 0.17 ^b	2.50 ± 0.28 ^{ab}	2.83 ± 0.41 ^{ab}	2.87 ± 0.31 ^{ab}	3.10 ± 0.45 ^a	2.43 ± 0.42 ^b
	6D	2.73 ± 0.19 ^b	2.93 ± 0.15 ^b	3.07 ± 0.16 ^{ab}	3.13 ± 0.12 ^{ab}	3.58 ± 0.43 ^a	2.65 ± 0.55 ^b

Data are expressed as mean ± standard deviation (SD), and all experiments were performed in triplicate. Significant differences in fungal growth across different TCW concentrations at specific time points are indicated by differing superscript letters ($p < 0.05$).

The fungi show a larger colony diameter on TCW-agar media than on the commercial Potato Dextrose Agar (PDA) media. The fungi grew very densely on PDA, forming a thick and compact colony within a limited circular area. In contrast, on TCW-agar media, the fungal growth was more spread out and less dense, covering a larger area overall. This indicates that while the fungi can grow more extensively in terms of surface area on TCW-agar, it tends to form a more concentrated and tightly packed colony on PDA.

However, using only the colony diameter to evaluate the effectiveness of the media may not provide accurate results. A larger diameter on TCW-agar does not necessarily imply that it is a better medium for fungal growth than PDA. The density of the fungal colony and the dry weight of the biomass are also important factors that need to be considered. The dense and compact growth on PDA suggests a higher biomass concentration in a smaller area, which might result in a higher dry weight of the fungal colony. In contrast, the more spread out and less dense growth on TCW-agar, might result in a lower dry weight despite covering a larger area.

Table 3 presents the dry weight of fungi on the 6th day of growth in different concentrations of TCW-agar media, with PDA used as

the control. The mycelial dry weight of all tested fungi was significantly higher on the commercial PDA media compared to the TCW-agar media. Among the various concentrations of TCW-agar media, the 100% concentration showed significantly greater dry weight. Despite the mycelial dry weight of fungi being lower on TCW-agar media compared to PDA, TCW-agar can still be considered a viable alternative medium for certain fungi based on the results. Tables 2 and 3 show that full-strength TCW-agar medium offers ideal fungal growth and biomass production conditions. Consequently, pure tender coconut water (100%) was selected for further optimization.

In a recent study exploring the potential of coconut water as a substitute for Sabouraud Dextrose Agar in cultivating *Aspergillus flavus*, various concentrations of coconut water ranging from 20% to 100% were examined. The findings revealed that concentrations of 70%, 80%, and 90% supported growth comparable to that observed with SDA (Widhorini et al., 2021). Therefore, Coconut water at higher concentrations provides the most favourable conditions for colony growth, possibly offering optimal nutrients and hydration levels.

Table 3. Dry weight measurement of fungi on different concentrations of TCW-agar media and PDA on 6th day

	Dry weight (mg)					
	20%	40%	60%	80%	100%	PDA
<i>Fusarium oxysporum</i>	3.4 ± 0.56 ^f	24.2 ± 1.65 ^e	41.2 ± 2.40 ^d	67.47 ± 3.32 ^c	80.90 ± 1.05 ^b	201.50 ± 1.47 ^a
<i>Aspergillus flavus</i>	12.0 ± 2.80 ^e	22.0 ± 1.08 ^{de}	27.0 ± 2.72 ^d	65.90 ± 3.35 ^c	143.10 ± 1.82 ^b	460.73 ± 9.56 ^a
<i>Mucor</i> sp.	8.7 ± 1.14 ^d	26.7 ± 1.18 ^d	78.0 ± 7.55 ^c	85.20 ± 3.16 ^c	162.93 ± 12.87 ^b	346.90 ± 12.41 ^a
<i>Penicillium chrysogenum</i>	2.9 ± 0.36 ^e	8.6 ± 0.56 ^e	21.4 ± 0.62 ^d	51.70 ± 3.57 ^c	72.90 ± 4.06 ^b	97.90 ± 5.24 ^a

Values are mean ± SD, all analyses were carried in triplicates and values with different superscript alphabets indicate significant differences $p < 0.05$

Effect of pH, Agar Concentration and Dextrose concentration on the Growth of Fungi

The use and availability of growth substrates, particularly glucose, play a critical role in predicting the growth rate and multiplication of fungi in laboratory cultures. Glucose, a simple sugar, serves as a primary energy source for many fungi, enabling their metabolic processes and growth (Sati & Bisht, 2006). This substantial concentration of sugars can significantly influence the growth dynamics of fungi when used as a culture medium. The high sugar content provides an abundant energy source, potentially leading to enhanced fungal growth and proliferation (Walker & White, 2017). When sugar concentrations exceed a certain level, they can inhibit fungal growth due to osmotic stress. High sugar levels cause water to be drawn out of fungal cells through osmosis, leading to dehydration and hindering essential metabolic processes. Additionally, excessive sugar can disrupt the osmotic balance within fungal cells, impacting their ability to regulate internal solute concentrations. Consequently, fungal growth may be stunted or completely halted in environments with high sugar concentrations (Gomar-Alba et al., 2015). Therefore, understanding the concentration and availability of glucose in the growth medium is essential for optimizing fungal cultivation. In this context, the total sugar concentration in tender coconut water (TCW) from coconuts aged 5 to 7 months is notably significant. Shayanthavi et al. (2024) reported that the total sugar content in TCW of this age varies between 46.28 and 63.58 mg/ml. Like all organisms, Fungi exhibit specific preferences for environmental conditions conducive to their growth and metabolic activity. One critical factor shaping fungal ecology and physiology is the pH of their surroundings. Different fungi have evolved to thrive within specific pH ranges that optimize their enzymatic processes, nutrient acquisition, and overall fitness. Generally, as the pH level moved closer to neutrality, the rate of fungal growth progressively declined. Eventually, at a pH of 7.0, the conditions became unsuitable for supporting any further fungal growth (Srivastava et al., 2011).

Taguchi design, implemented using Minitab 17, optimized factors like agar concentration, dextrose concentration, and pH of the culture media through an L9 orthogonal array, allowing systematic evaluation across nine experiments. This approach

identified the optimal conditions for a reliable and efficient culture medium composition. (Pundir et al., 2016). As per the experiments conducted, Figures 1-4 display the graphical representation of the data showing how the diameter of fungal growth varies with formulated TCW media at specific time points. The figures are in the following order: Figure 1 (*Fusarium oxysporum*), Figure 2 (*Aspergillus flavus*), Figure 3 (*Mucor* sp.), and Figure 4 (*Penicillium chrysogenum*).

The observations made on the 3rd, 4th, 5th, and 6th day indicate that all the chosen fungi exhibited growth development. F5 (1% agar, 1% dextrose, pH of 6.6) exhibited a significantly larger diameter of *Fusarium oxysporum* growth on the 3rd, 4th, 5th, and 6th days. F5 recorded diameters of 4.03 cm, 5.52 cm, 6.93 cm, and 7.60 cm on the respective days. In contrast, F8 (1.5% agar, 1% dextrose, and pH of 4.6) showed diameters of 3.75 cm, 5.10 cm, 6.75 cm, and 7.42 cm on the same days. Despite F5 showing a consistently higher diameter of fungal growth, F8 demonstrated a significantly greater dry weight of fungal biomass based on dry weight measurement (Table 4). The dry weight for F8 was 197.80 mg, whereas for F5, it was 124.40 mg. This indicates that while F5 promoted more extensive surface growth, F8 facilitated a denser and possibly more substantial fungal mass. Therefore, considering the higher dry weight, which is an important indicator of fungal biomass, the F8 medium (1.5% agar, 1% dextrose, and pH of 4.6) was determined to be the best formulation for cultivating *Fusarium oxysporum*. This suggests that F8 provides a more favourable environment for the development and accumulation of *Fusarium oxysporum* biomass.

Although *Aspergillus flavus* showed significantly larger colony diameters on medium F8 during the 3rd, 4th, 5th, and 6th days, the final dry weight of the fungus was highest on medium F5 (235.10 mg). Therefore, medium F5, which contains 1% agar, 1% dextrose, and has a pH of 6.6, is considered the most suitable for promoting fungal biomass accumulation, while medium F8 which contains 1.5% agar, 1% dextrose, and has a pH of 4.6 is better for extensive surface growth. For *Mucor* sp., the colony diameter on the 3rd day was significantly larger on medium F8 (6.45 cm). However, by the 5th day, all eight formulated media displayed significantly similar growth in terms of diameter. On the 6th day, media F2, F3, F4, F7, and F8 showed significantly higher diameters than the others. Despite this, the dry weight measurement indicated that medium F4 (1% agar, 0% dextrose, pH of 5.6)

supported the highest growth, with a dry weight of 174.90 mg, surpassing the other formulated media.

The colony diameter of *Penicillium chrysogenum* on the 3rd day was similar for F2, F3, F4, F5, F6, F7, and F8. By the 6th day, F4 showed a significantly larger colony diameter (3.65 cm), and a significantly higher dry weight (84 mg). Therefore, based on both colony diameter and dry weight measurements, the F4 medium (1% agar, 0% dextrose, pH 5.6) was the most effective for the

growth of *Penicillium chrysogenum*. Overall, the study highlights that colony diameter evaluates how effectively a medium promotes rapid fungal growth and spread, while fungal dry weight reveals the medium's ability to support substantial biomass accumulation. While surface growth can provide initial insights, the dry weight offers a more comprehensive indicator of biomass accumulation and overall fungal development.

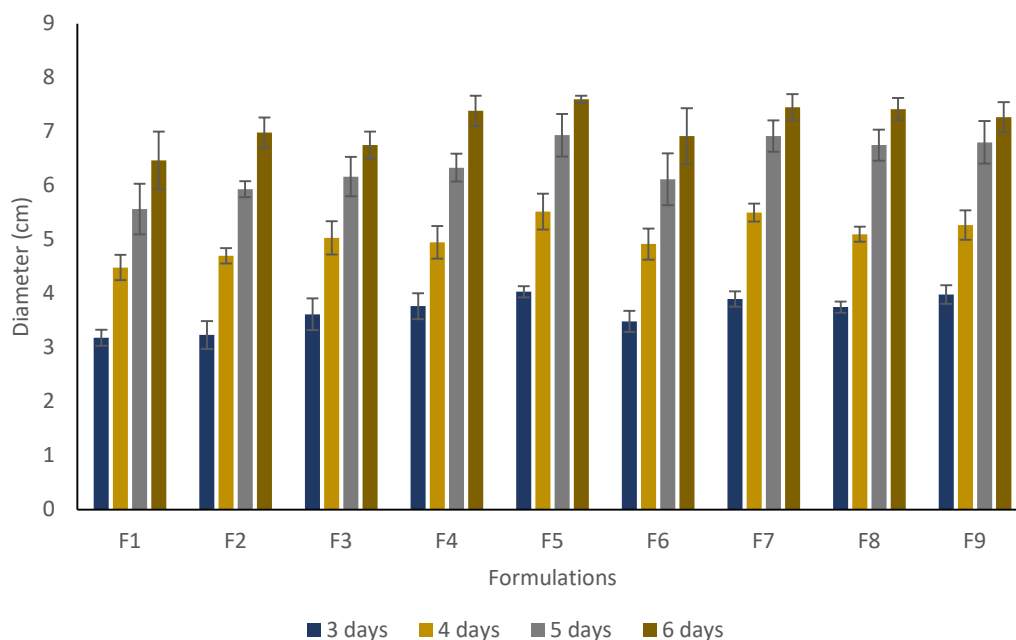


Figure 1. *Fusarium oxysporum* growth on the formulated media was measured on the 3rd, 4th, 5th and 6th day

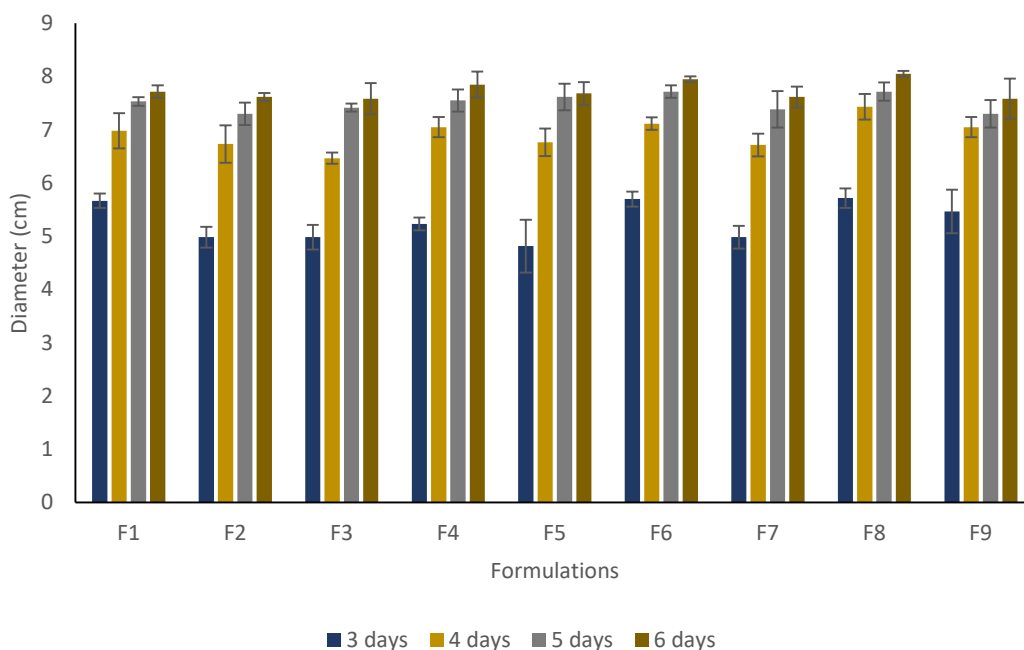


Figure 2. *Aspergillus flavus* growth on the formulated media measured on the 3rd, 4th, 5th and 6th day

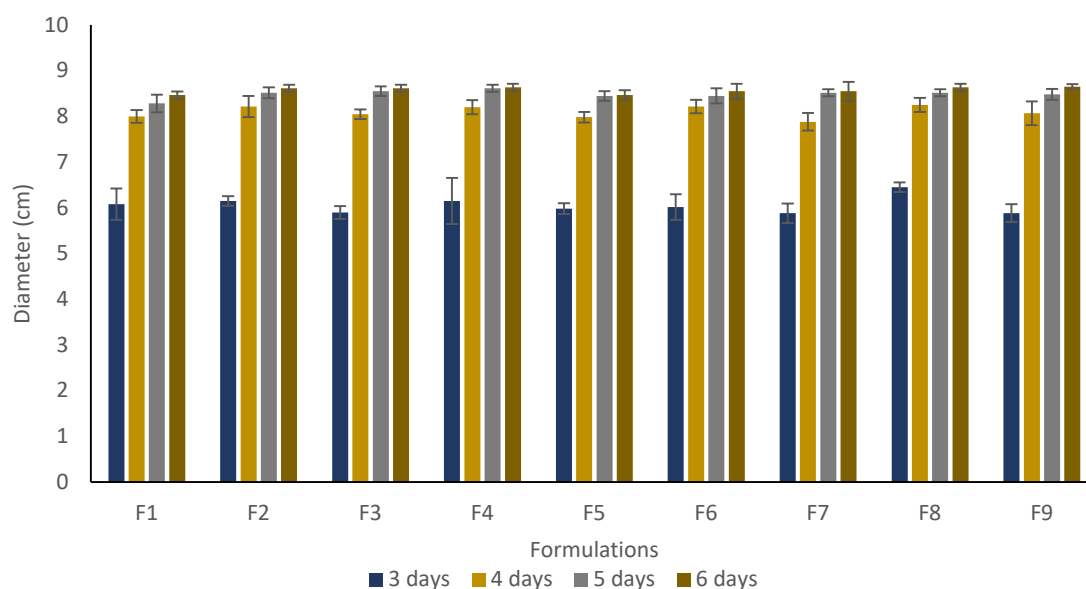


Figure 3. *Mucor* sp. growth on the formulated media measured on the 3rd, 4th, 5th and 6th day

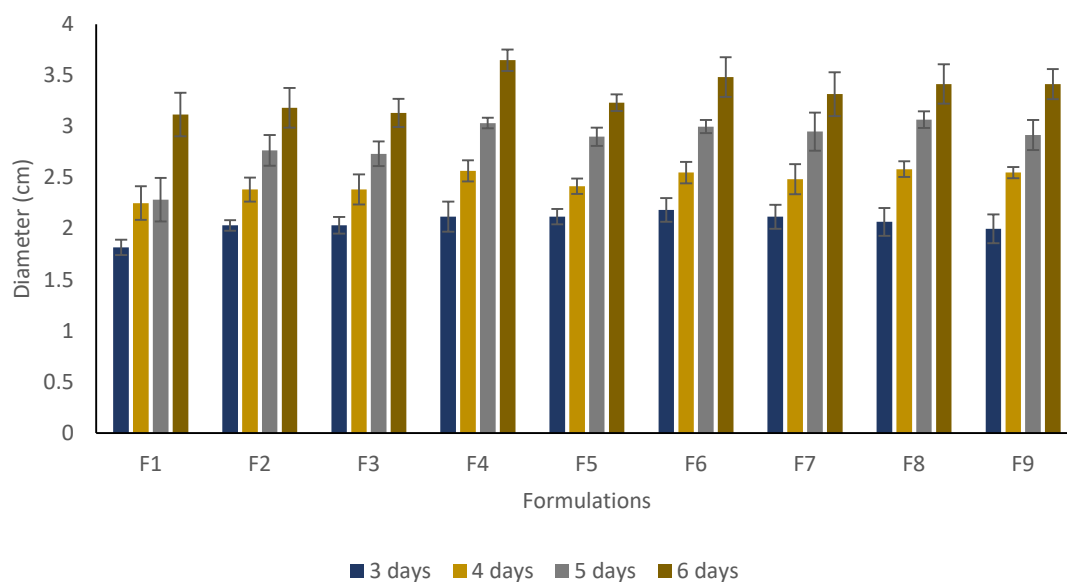


Figure 4. *Penicillium chrysogenum* growth on the formulated media measured on the 3rd, 4th, 5th and 6th day

According to Tables 3 and 4, the dry weight measurements of *Fusarium oxysporum*, *Aspergillus flavus*, *Mucor* sp., and *Penicillium chrysogenum* were higher on PDA compared to all the formulated media. Fungal colony diameter was consistently larger on TCW compared to PDA, indicating that TCW may support more extensive fungal colony expansion. However, the fungal biomass, as measured by dry weight, was greater on PDA than on TCW, suggesting that PDA is more conducive to fungal growth and development in terms of overall biomass accumulation. This implies that TCW is favourable for promoting fungal colony spread, while PDA is more effective for enhancing fungal biomass. Therefore, the choice of medium can influence the growth

characteristics of fungi, with TCW supporting larger colonies and PDA facilitating higher fungal biomass. However, the test microorganisms in this study also grew well on the formulated media. Tender coconut water (TCW), a locally available resource, was used effectively in these formulations. TCW supports the growth of a variety of microorganisms, making it a suitable and cost-effective culture medium for microbiological applications. The performance of TCW-agar suggests that it could facilitate continued microbiological research, particularly under conditions of economic constraint.

Table 4: Dry weight measurement of fungi on formulated media

	Dry weight (mg)			
	<i>Fusarium oxysporum</i>	<i>Aspergillus flavus</i>	<i>Mucor</i> sp.	<i>Penicillium chrysogenum</i>
F1	40.90 ± 2.46 ^f	121.60 ± 5.75 ^e	98.30 ± 5.37 ^{cd}	33.40 ± 2.83 ^e
F2	40.97 ± 2.14 ^f	97.30 ± 6.63 ^f	92.54 ± 6.15 ^{cd}	35.30 ± 2.69 ^e
F3	42.10 ± 4.30 ^f	149.80 ± 5.77 ^d	84.80 ± 3.97 ^d	72.40 ± 3.57 ^{bc}
F4	92.10 ± 1.96 ^c	210.70 ± 9.35 ^b	174.90 ± 4.25 ^a	84.00 ± 4.80 ^a
F5	124.40 ± 4.61 ^b	235.10 ± 5.56 ^a	129.30 ± 8.01 ^b	75.00 ± 4.08 ^{ab}
F6	51.90 ± 3.60 ^e	125.80 ± 3.66 ^e	123.40 ± 6.31 ^b	39.25 ± 4.70 ^{de}
F7	132.10 ± 2.80 ^b	150.23 ± 3.69 ^d	102.90 ± 2.64 ^c	63.00 ± 4.85 ^c
F8	197.80 ± 3.22 ^a	216.33 ± 4.05 ^b	105.60 ± 3.74 ^c	62.41 ± 3.36 ^c
F9	70.80 ± 2.24 ^d	172.60 ± 3.54 ^c	94.70 ± 4.16 ^{cd}	49.24 ± 4.67 ^d

Data are presented as mean ± standard deviation (SD), and each analysis was conducted in triplicate. Variations indicated by distinct superscript letters reflect significant differences at $p < 0.05$.

In a study, coconut water dextrose broth and coconut water dextrose agar medium were used to culture *Emericella nidulans*. Potato dextrose broth yielded significantly ($p < 0.01$) greater fresh and dried mycelial pellets compared to those from coconut water dextrose broth. *E. nidulans* exhibited higher growth when grown on coconut water dextrose agar, while on potato dextrose agar, its mycelial growth was slower, and its diameter was narrower. Throughout the observation period, coconut water dextrose agar at pH 6 consistently showed the highest mycelial growth (Sibounnavoung et al., 2009). In another study using banana peel as an alternative medium, *Aspergillus fumigatus* and *Mucor hiemalis* were grown on waste banana peel agar (BPA) and broth (BPB), showing comparable growth and biomass production to commercial malt extract media (MEA and MEB). The moulds exhibited slight variations in spore sizes and hyphal growth, with minor reductions in biomass on BPB compared to MEB (Essien et al., 2005). Another study clearly indicates that the natural medium containing tomato juice (tomato juice agar medium) over Czapek's agar for cultivating tested fungal species such as *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Penicillium corylophilum*, *P. funiculosum*, and *Rhizopus stolonifer*. When comparing the growth of these species on both media, it was observed that tomato juice agar significantly enhanced radial growth, particularly in *R. stolonifer*, which showed a remarkable increase at pH 5. Additionally, *A. niger* demonstrated optimal growth on tomato juice agar at pH 3.7, highlighting its effectiveness as a versatile substrate for a wide range of fungal species (Abdel-Mallek et al., 1995).

In a study, watermelon peel waste (WPW) was tested for its potential as a fungal growth medium, aiming to replace expensive options like Potato Dextrose Agar (PDA). The optimal growth medium was identified through Taguchi design by blending 500 g/L of fresh WPW with 10 g/L of dextrose. Dry biomass measurements for various fungi demonstrated significant increases on WPW-derived medium compared to Potato Dextrose medium and Czapek's Dox medium. Additionally, WPWDA

medium exhibited similar physicochemical properties to PDA and CzDA, indicating its effectiveness as an eco-friendly and cost-efficient alternative for fungal growth (Hasanin & Hashem, 2020).

Over the years, various studies have explored alternative natural media for fungal cultivation as substitutes for commercial media. Researchers have been investigating the potential of using cost-effective and sustainable resources to develop nutrient-rich growth media for fungi. These efforts aim to reduce the reliance on expensive traditional media like Potato Dextrose Agar (PDA), by finding natural materials that can provide similar or enhanced growth conditions for a wide range of fungal species.

4. Conclusion

Coconut water contains a variety of nutrients essential for fungal growth, including sugars, vitamins, minerals, and amino acids. These nutrients provide a conducive environment for the proliferation of many fungal species. In contrast to conventional laboratory media such as Potato Dextrose Agar (PDA), coconut water is a more cost-effective and accessible option, particularly in tropical regions where coconuts are plentiful. Coconut water is biodegradable and environmentally friendly, making it a sustainable choice for fungal culture compared to synthetic media.

Based on this study, while colony diameter provides a quick visual indication of growth, dry weight is a more reliable measure of fungal biomass, which is crucial for applications requiring substantial fungal production. Fungi grown on TCW-agar exhibit sparse growth due to its minimal nutrient composition, while those on PDA display compact and denser growth owing to its rich nutrient content, particularly carbohydrates from potato extract and dextrose, supporting vigorous fungal proliferation. Although TCW may not fully replace PDA, it demonstrated potential as a natural, cost-effective, and supportive medium. The optimal media formulation varies significantly between fungal species, reflecting their unique growth requirements.

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