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Phyllanthus Nutrient Agar (PNA) as an Alternative Culture Media for Colletotrichum Species

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Abstract

Culture media or growth media is a medium to isolate and grow microorganisms such as fungal pathogens and bacteria. The identification of plant pathogen requires massive usage of culture media as this is the most common method for pathogen isolation and identification. In developing countries, the high cost of culture media supply has restricted many practical classes in certain institutions and limits the studies on scientific research. Besides, Potato Dextrose Agar (PDA), which is known as universal media for fungi isolation could inhibit spore production in some fungi due to its high nutrient content. *Colletotrichum* spp. is the causal agent of anthracnose disease that widely infects tropical and subtropical crops such as chilli, black pepper, rubber, tomato and many more. The identification of *Colletotrichum* species often requires the observation of distinct morphological features, especially spore characteristics which may not be easily visible on nutrient-rich media like PDA. This study aims to evaluate the potential of a novel, cost-effective culture medium which is Phyllanthus Nutrient Agar (PNA) that formulated from *Phyllanthus emblica* (Indian gooseberry), a locally abundant and historically significant medicinal plant in Malaysia. The effectiveness of PNA as culture media for *Colletotrichum* species were compared with commercial culture media for plant pathogen which were Potato Dextrose Agar (PDA) and Tomato Juice Agar (TGA). The results indicated that two species of *Colletotrichum* namely *Colletotrichum gloeosporioides* and *Colletotrichum truncatum* were able to grow on PNA and the culture were suitable for morphological identification of the species. These findings suggest that PNA is a promising alternative culture medium for the isolation and study of *Colletotrichum* species which offering a sustainable and affordable option for fungi isolation and identification.

1. Introduction

Culture media are essential for isolating and cultivating microorganisms or plant pathogens such as fungi and bacteria [1]. Suitable culture media for fungal isolation and identification are crucial, as the type of medium can influence the morphological characteristics of the fungus [2]. The nutritional composition of culture media plays a significant role in supporting fungal growth, particularly through key elements such as carbohydrates and nitrogen [3]. However, the use of commercial culture media in large-scale laboratory work incurs high costs, prompting researchers to explore more affordable alternative media.

Natural media are formulated from substrates such as corn, potato, tomato, woody stems, and oatmeal. Currently, Potato Dextrose Agar (PDA) is widely regarded as a universal culture medium for fungal pathogens due to its ability to promote mycelial growth across a broad range of fungi. However, several studies have reported a limitation of PDA, where its high carbon content, particularly glucose and starch, may promote excessive vegetative growth while suppressing sporulation in certain fungal taxa [4,5]. Since sporulation is important for fungal identification, especially in forming pigmentation and colony characteristics, this limitation can affect the accuracy of diagnosis. Therefore, there is a need to explore alternative natural media that not only support mycelial growth but also enhance sporulation and morphological differentiation of fungal pathogens.

Phyllanthus emblica L., commonly known as Indian gooseberry, is a member of the Euphorbiaceae family and is widely distributed in tropical and subtropical regions, including Malaysia, Indonesia, and China [6]. The plant is characterized by a small to moderate-sized tree with greenish-grey bark, yellow flowers, and fleshy greenish-yellow fruits upon ripening. The fruit of *P. emblica* is rich in bioactive compounds such as vitamin C, phenolic compounds, tannins, and organic acids, which contribute to its strong antioxidant and antimicrobial properties. These biochemical constituents may influence fungal growth behavior, including colony morphology and sporulation, making it a potentially suitable substrate for developing natural culture media.

In addition, plant-based media derived from natural extracts have been explored in previous studies as cost-effective alternatives to commercial media, with some showing comparable or improved performance in supporting fungal growth and sporulation.The aqueous extract of *P. emblica* has been reported to contains antimicrobial activity against certain bacteria such as *Escherichia coli* and *Klebsiella pneumoniae* [7]. However, the application of *P. emblica*-based agar as a culture medium for fungal pathogens remains limited and underexplored. This highlights a research gap in evaluating its effectiveness as an alternative medium.

Colletotrichum spp. is one of the most destructive fungal pathogens causing anthracnose disease. This pathogen commonly infects tropical and subtropical crops, including ornamental plants [8]. It is also associated with postharvest infections, particularly during storage, leading to significant losses [9]. Anthracnose is considered a serious disease as it reduces both yield and market value. For example, the development of blackish lesions on fruits significantly decreases their aesthetic quality and commercial price [10]. In Malaysia, crops such as chili, mango, and banana are frequently affected by this disease [11]. The most associated species include *Colletotrichum gloeosporioides* and *Colletotrichum truncatum* [12].

Therefore, this study was conducted to evaluate the potential of PNA as an alternative culture medium for supporting the growth and morphological development of fungal pathogens, particularly *Colletotrichum* spp. The aim of this study is to assess the effectiveness of PNA compared with conventional PDA and Tomato Juice Agar (TJA) for fungal cultivation. Specifically, the objectives are (i) to evaluate the mycelial growth of *Colletotrichum* spp. on PNA, (ii) to assess the ability of PNA to enhance sporulation and morphological characteristics, and (iii) to compare the overall performance of PNA with PDA and (TJA) as a standard culture medium.

2. Materials and Methods

2.1 Fungal Sample

The pure cultures of *C. gloeosporioides* and *C. truncatum* were obtained from Plant Pathology laboratory of Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, Malaysia. A fragment of the fungal colony was taken from the cultures and transferred onto PDA media. The PDA media were then incubated in incubator at 28 °C for 7 days to encourage the growth of the fungal pathogen. The morphological characteristics of the fungal pathogen were identified once again to verify the species of the pathogen.

2.2 Media Preparation

Three types of culture media were utilized in this study which are Potato Dextrose Agar (PDA), Tomato Juice Agar (TJA), and Phyllanthus Nutrient Agar (PNA). Potato Dextrose Agar was prepared by dissolving 35 g of commercial PDA powder (Merck, Germany) in 1 L of distilled water followed by heating and stirring until fully dissolved. For TJA, 350 g of ripe tomatoes were blended and filtered through a double layer of sterile muslin cloth to obtain 200 mL of purified juice which was then mixed with 1 L of distilled water, 30 g of Nutrient Agar (Oxoid, UK), and 10 g of D-glucose (Sigma-Aldrich, USA). For PNA, 100 g of fresh, healthy *Phyllanthus* leaves were washed, chopped

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and homogenized with 100 mL of sterile distilled water using a sterile blender. The mixture was filtered through sterile muslin cloth, and the extracted volume was adjusted to 200 mL with sterile distilled water. This extract was then combined with 1 L of distilled water, 30 g of Nutrient Agar and 10 g of D-glucose. All media were mixed using a magnetic stirrer, heated until all components were completely dissolved and sterilized by autoclaving at 121 °C for 20 minutes.

After autoclaving, the media were allowed to cool to approximately 50 °C before being poured into sterile Petri dishes under aseptic conditions. Prepared agar plates were stored at 4 °C and used within two weeks to maintain media integrity and sterility.

2.3 Inoculation of the Pathogen onto Media

Approximately 5 mm^2 of actively growing fungi that previously obtained from pure culture were transferred at the centre of sterile petri dishes containing three different growth media (i) PDA (ii) TJA (iii) PNA. For each culture media, five replicates of fungal isolates were prepared for each fungus species. The pathogens were allowed to grow in incubator at 28 ℃ for 7 days. The important features for *Colletotrichum* identification such as colony diameter, colony color, conidia shapes and sporulation were recorded.

2.4 Sporulation Assessment

The level of sporulation was determined through microscopic observation and conidial counting using a haemocytometer. Small mycelial fragments (approximately 5mm × 5 mm) were aseptically taken from the active margins of 7-day-old fungal cultures grown on each test medium. These fragments were suspended in 5 mL of sterile distilled water and vortexed gently to dislodge the conidia. The spore suspension was filtered through sterile muslin cloth to remove hyphal debris. A 10 µL of the filtered suspension was then loaded onto a haemocytometer and the conidia were counted under a light microscope at 40× magnification. Conidia counts (sporulation) were conducted in five selected grid areas and the average number of conidia per mL was calculated using the standard haemocytometer formula. Based on the conidia counts, sporulation was categorized into three levels: Low ($<1 \times 10^6$ conidia/mL), Moderate (1×10^6 to 5×10^6 conidia/mL) and High ($>5 \times 10^6$ conidia/mL).

3. Results and Discussion

3.1 Effect of Different Culture Media on Colony Diameter of *Colletotrichum* Species

Table 1 showed the colony diameter of *C. gloeosporioides* and *C. truncatum* on different culture media. The result indicated that *C. gloeosporioides* recorded the highest mean of diameter on PDA (72.333±1.607) followed by PNA (50.50±8.05) and TJA (47.333±1.607). Meanwhile, *C. truncatum* produced a quite similar colony diameter on all culture media; PDA (47.83±5.30), TJA (43.33±7.82) and PNA (42.833±0.289).

Table 1 *Colony diameter of C. gloeosporioides and C. truncatum on different culture media*

Culture Media	*C. gloeosporioides* (Mean ± SD)[1]	*C. truncatum* (Mean ± SD)[1]
PDA	72.333 ± 1.607[a]	47.83 ± 5.30[a]
TJA	50.50 ± 8.05[b]	43.33 ± 7.82[b]
PNA	47.333 ± 1.607[c]	42.833 ± 0.289[c]

[1]Mean ± standard deviation followed by the same letter are not significantly different ($p<0.05$) according to Tukey's test.

The results of this study demonstrated that all tested *Colletotrichum* species exhibited optimal growth on PDA, confirming its efficacy as a standard medium for fungal cultivation. This observation is consistent with findings by Majumdar and Mandal [13] who reported that the colony diameters of various *Colletotrichum* species were significantly larger on PDA compared to other tested media, likely due to the high carbohydrate content and rich nutrient profile provided by the potato infusion and dextrose. Potato Dextrose Agar also has long been recognized for its ability to support rapid fungal growth due to its balanced composition of simple sugars and polysaccharides which are readily assimilated by filamentous fungi [14]. Tomato Juice Agar also demonstrated the potential to sustain fungal growth at moderate levels. This aligns with the observations by Okoye and Abba [15] who reported that TJA could provide sufficient nutrients for fungal growth such as sugars, organic acids, and vitamins.

Interestingly, PNA also showed potential in supporting the growth of *Colletotrichum* species indicating its applicability as an alternative plant-based medium. This may be attributed to the phytochemical composition of the leaves which consist of substantial amounts of carbohydrates, organic acids, and phenolic compounds [16]. Carbohydrates are essential as primary carbon sources for fungal metabolism and biomass accumulation. Moreover, the presence of trace minerals and natural growth stimulants in *Phyllanthus* extracts may contribute to fungal development. While PDA remains superior in terms of colony expansion due to its well-established

nutrient composition, the ability of PNA to support fungal growth underscores its potential as a low-cost, plant based alternative culture medium. Besides, the use of readily available botanical sources such as *Phyllanthus emblica* offers an economical substitute to commercial media particularly in resource-limited laboratories or field-based studies

3.2 Effect of Different Culture Media on Colony Color of *Colletotrichum* Species

Figure 1 showed the effect of different culture media on colony color of *Colletotrichum* species. On PDA, *C. gloeosporioides* produced whitish to orange colony while *C. truncatum* produced grey to blackish colony. On TJA, *C. gloeosporioides* produced salmon pink colony with some white pigmentation and *C. truncatum* produced cloudy white colony with creamy yellow pigmentation. On PNA, *C. gloeosporioides* produced creamy yellow colony with light yellowish pigmentation while *C. truncatum* produced dull brown colony with white and grey pigmentation.

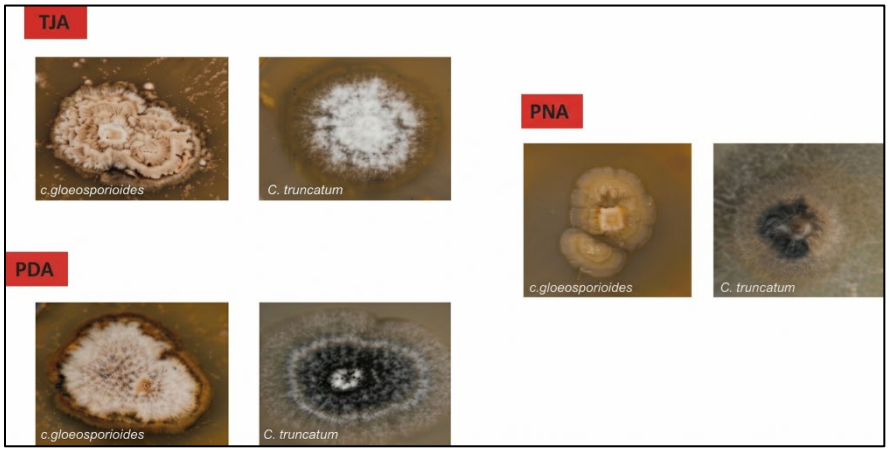

Fig. 1 *Effect of different culture media on colony color of Colletotrichum species*

In this study, the type of culture medium was found to significantly influence the colony pigmentation of *Colletotrichum* species. This finding aligns with previous reports by Lokare and Fatima [17], who demonstrated that the pigmentation of *Colletotrichum* species is highly responsive to the nutrient composition and pH of the growth medium. Fungal pigmentation is also associated with the production of secondary metabolites such as melanin, which contributes to colony colour and structural differentiation. Melanin is a key component in fungal cell walls and is involved in protection against environmental stress and maintenance of morphology characteristics.

In *Colletotrichum* spp., melanin biosynthesis is tightly regulated and linked to developmental processes such as appressorium formation and colony differentiation [18]. Notably, colonies grown on PNA exhibited pigmentation patterns that closely resembled the diagnostic characteristics of *C. gloeosporioides* and *C. truncatum* as described in the taxonomic frameworks of Weir et al. [19] and Wang et al. [20]. The improved pigmentation observed on PNA may be attributed to the presence of bioactive compounds in Phyllanthus emblica, including phenolics, tannins, and organic acids, which can act as mild stress factors that stimulate secondary metabolite production [21]. Previous studies have shown that environmental or nutritional stress can enhance melanin synthesis and sporulation in fungi, thereby improving morphological differentiation as discuss by Pedras et al. [22]. In contrast, studies have shown that fungal cultures grown on PDA may exhibit reduced or inconsistent pigmentation due to variations in nutrient composition, particularly limited copper availability which directly affects pigment-producing enzymes such as laccases [23] . These findings highlight the limitation of PDA in expressing stable diagnostic characteristics necessary for accurate fungal identification.

3.3 Effect of Different Culture Media on Conidia Shapes of *Colletotrichum*

Figure 2 showed that the *Colletotrichum* species produce similar conidia shapes on all types of media with *C. gloeosporioides* produced cylindrical conidia while *C. truncatum* produced falcate or curved conidia.

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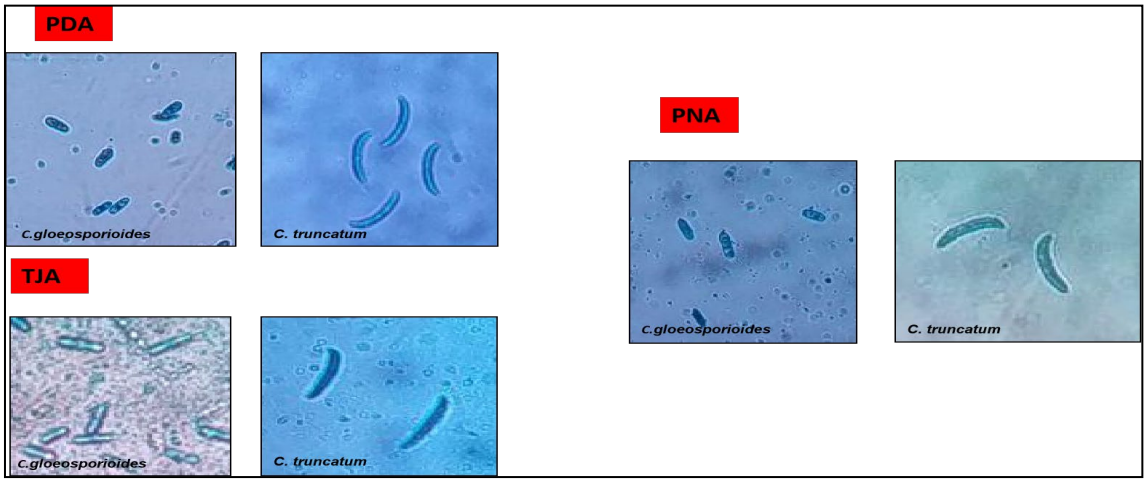

Fig. 2 *Effect of different culture media on conidia shapes of Colletotrichum*

This study indicated that the conidia shape of *Colletotrichum* species were not influenced by the type of culture media and has been supported by the similar finding found by El-Marzoki and Shaban [24]. The conidia shapes of the *Colletotrichum* on PNA, PDA and TJA are similar with the description of conidia shapes of *C. gloeosporioides* by Choi et al. [25] and *C. truncatum* by Tuluhong et al. [26]. The stable conidial morphology observed in this study suggests that conidial shape is largely genetically determined and less affected by external nutritional conditions. Although different media may influence colony colour, mycelial growth rate, sporulation, and pigment production, the basic conidial structure generally remains conserved within a species. Comparable observations were also reported in recent studies by Guarnaccia et al. [27] on *Colletotrichum* associated with citrus, ornamental plants, and legumes, where conidial morphology remained relatively unchanged across different artificial media.

3.4 Effect of Different Culture Media on Sporulation of *Colletotrichum* Species

Table 2 indicated that PNA could induced better sporulation for the *Colletotrichum* species compared to PDA and TJA.

Table 2 *Effect of different culture media on sporulation of Colletotrichum species*

Media	*Colletotrichum* species	Sporulation
PDA	*C. gloeosporioides*	Moderate
	C. truncatum	Moderate
TJA	*C. gloeosporioides*	High
	C. truncatum	Moderate
PNA	*C. gloeosporioides*	High
	C. truncatum	High

This finding underscores the critical role of culture media not only in supporting fungal colony development but also in modulating sporulation efficiency. Joshi et al. [28] demonstrated that *C. capsici* cultured on carrot dextrose agar and potato carrot agar exhibited significantly enhanced sporulation compared to those grown on conventional PDA emphasizing the potential of plant extract-based media in promoting fungal reproductive development. In the present study, the improved sporulation observed on PNA may be attributed to its moderately rich and balanced nutrient profile which appears to favor reproductive differentiation over excessive vegetative growth. As suggested by Rao et al. [29], highly nutrient rich media like PDA are optimal for supporting rapid mycelial expansion but may inadvertently suppress sporulation due to the lack of nutrient stress or environmental cues typically required to trigger spore formation.

4. Conclusions

The identification and systematic classification of fungal species still largely depend on morphological characteristics including colony diameter, pigmentation and conidia shapes. Therefore, the choice of an

appropriate culture medium is critical for optimizing both the growth and sporulation required for accurate taxonomic identification. While PDA remains widely used due to its ability to support vigorous fungal growth, its nutrient rich composition may suppress fungal sporulation that potentially hinders diagnostic accuracy. The present study also demonstrates that PNA, a plant-based culture media, not only sustains mycelial growth but also significantly enhances sporulation of *Colletotrichum* species indicating its potential as a viable alternative to conventional media. The moderate nutrient balance of PNA may provide stress cues conducive to sporulation thereby supporting its utility in fungal identification. Future research should expand the evaluation of PNA across a broader spectrum of phytopathogenic fungi including *Fusarium*, *Phytophthora*, *Alternaria* and others to further validate its efficacy and versatility as selective culture medium in mycological studies.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Muhammad Arif Aiman Kamarul Bahrin, Nuraini Mohd Noor;* ***data collection:*** *Muhammad Arif Aiman Kamarul Bahrin;* ***analysis and interpretation of results:*** *Muhammad Arif Aiman Kamarul Bahrin, Nuraini Mohd Noor;* ***draft manuscript preparation:*** *Muhammad Arif Aiman Kamarul Bahrin, Siti Nur Anisah Aani. All authors reviewed the results and approved the final version of the manuscript.*

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