Comparison between Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) with Banana Peel (BP) for studying fungi in the air by settle-plate method.

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***Abstract*- The present study aimed to compare the efficacy of Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) supplemented with Banana Peel (BP) as a substrate for studying airborne fungi using the settle-plate method. Airborne fungi pose significant health risks, and understanding their presence and diversity is crucial for effective control and prevention measures. Settle-plate sampling was employed to collect airborne fungal spores onto SDA and PDA media supplemented with BP. The BP addition was chosen due to its potential as a nutrient source for fungi, which may enhance their growth and promote their detection. The settle-plate method involved exposing the culture media to the air for a specified period, allowing the settling of airborne particles, including fungal spores. Settle-plates were utilized for each medium type, and the experiments were conducted in various indoor and outdoor environments with different levels of fungal contamination. After incubation, the colonies were enumerated and identified based on their morphological characteristics. The results revealed that both SDA and PDA supplemented with BP effectively supported the growth of airborne fungi. However, distinct differences were observed in terms of colony morphology and fungal diversity. SDA exhibited better selective properties for fungal growth, allowing the identification of a wide range of fungal species. On the other hand, PDA supplemented with BP exhibited a higher colony count, indicating enhanced fungal growth. Notably, the use of BP as a substrate provided additional advantages in terms of promoting fungal growth. The banana peels organic composition likely contributed to the nutritional enrichment of the culture media, leading to increased fungal colonization and improved detection sensitivity.**

***Keywords*- SDA, PDA with Banana Peel, Fungi, Settle Plate Method, Quality control, Environment, Indoor & Outdoor, Air sample.**

1. INTRODUCTION

If the human eye possessed the ability to perceive images with the same level of detail as a light microscope, we would be able to detect bacteria and fungi abundantly present in various settings. These microorganisms thrive in the air, water, food, soil, as well as within the tissues of plants and animals. Any environment capable of sustaining life harbors a population of bacteria or fungi. Bacteria, being prokaryotes, benefit from their relatively simplistic nature. On the other hand, fungi, as eukaryotes and therefore genetically more intricate, proliferate at such a rapid pace that numerous generations can arise within a short span of time[1]. The banana holds a prominent position among global fruit crops and is cultivated in more than 122 nations across the world. Typically, bananas have a brief period of freshness and begin to degrade shortly after being harvested. The primary utilized component of the banana is its fruit flesh, while the outer peel is primarily employed as animal feed and organic fertilizer. Recently, the banana peel has found application in diverse industrial sectors, such as the production of biofuels, bio-absorbents, pulp and paper, cosmetics, activities related to energy, organic fertilizer, environmental remediation and processes associated with biotechnology[2]. The occurrence of fungi as disease-causing agents in humans is on the rise and we continually come across new and less common species. The identification of these species based on their growth on conventional culture media can be difficult due to the lack of distinctive characteristics. To overcome this challenge, a cost-effective and locally available alternative medium, known as banana peel culture, mimics the natural environment where these fungi thrive. This medium proves valuable in facilitating the development of reproductive structures that possess characteristic features aiding in the identification process[3]. Airborne fungi play a significant role in causing fungal infections in humans and animals. To assess the presence of these fungi, a settle plate method was employed by exposing 90 mm plates containing Saboraud Dextrose agar and Potato dextrose agar with Banana Peel to the air for a duration of 2 hours. Subsequently, the plates were placed in a 37°C incubator and visually examined daily for any observable fungal growth. The identified fungal colonies were then classified at the genus level based on their morphological characteristics, following standard procedures. The presence of airborne fungi in the atmosphere poses a potential risk to public health, as they encompass a wide range of pathogenic and allergenic spores that can contaminate indoor environments including homes, offices, and hospitals. It has been documented that over 180 genera of airborne fungi, distributed worldwide, are associated with allergies and pose a

serious threat of infections in humans and animals. Considering that indoor fungi are primarily derived from outdoor sources, it is important to recognize the potential impact of an increase in outdoor fungi on the elevated risk of fungal diseases. Airborne fungi from various groups act as significant.

2. MATERIALS & METHODS

2.1 Materials

Sabouraud Dextrose Agar (SDA) plate, Sabouraud Dextrose Agar (SDA) tubes, Potato Dextrose Agar (PDA)with banana peel, Deionized water, Agar, Ripe banana peel, Potato Dextrose, Petri dish/week, Autoclaved distilled water, Lactophenol cotton blue, Sterile cover slips.

2.2 Methods

2.2.1 Preparation of Potato Dextrose Agar with Banana Peel (PDA with BP).

The composition of Potato Dextrose Agar with Banana Peel involves combining dehydrated Potato Infusion, Dextrose, and ripe Banana Peel to promote abundant fungal growth. Agar is then added as a solidifying agent. To prepare the potato infusion, 200 grams of sliced, Potatoes are boiled in 1 litre of distilled water for 30 minutes. The resulting mixture is filtered through cheesecloth, with the liquid collected and saved as potato infusion. Alternatively, a commercially available dehydrated form can be used. The potato infusion is then mixed with ripe Banana Peel, Dextrose, Agar, and water. Later the mixture is boiled until dissolved. Subsequently the mixture is autoclaved for 15 minutes at a temperature of 121°C and 15 psi pressure. Portions of 20-25 ml are dispensed into sterile 15 × 100 mm petri dishes. The final pH is adjusted to approximately 5.6 ± 0.2[4].

2.2.2 Preparation of Saboraud Dextrose Agar (SDA).

Sabouraud Dextrose Agar, a longstanding and widely employed medium, serves as a primary choice for the isolation and cultivation of fungi. It exhibits selectivity in isolating fungi from diverse environmental sources like air and soil. Moreover, it enables the maintenance of pure fungal cultures and supports the growth of fungi, allowing for the differentiation and identification of various species, particularly dermatophytes, based on their visual characteristics and pigmentation. Sabouraud Dextrose Agar comprises hydrolysed animal tissue extracts (peptones), which serve as a rich supply of amino acids and nitrogenous compounds essential for the nourishment and proliferation of fungi and yeasts. Dextrose is included as a source of energy and carbon. Agar functions as the solidifying agent in the medium. Combine all the specified ingredients in approximately 900 ml of deionized water. Adjust the pH to 5.6 using hydrochloric acid and make sure the final volume reaches 1 litre. Heat the mixture until it reaches boiling point ensuring complete dissolution of the medium. Autoclave the solution at a temperature of 121ºC for a duration of 15 minutes. Allow the solution to cool down to approximately 45 to 50°C and pour it into petri dishes or tubes to create slants. The final pH at 25ºC should be 5.6 ± 0.2. To inoculate Sabouraud agar plates, one can either streak the surface, following the same method as with standard bacteriological media, or expose the medium to the surrounding air [5].

2.2.3 Settle Plate Method.

Air sampling serves as a valuable tool for scientific research, quality assurance, and educational purposes. Methods utilized for microbial air sampling can be categorized into two groups: passive and active. The passive method involves measuring the rate at which microorganisms settle on surfaces. It relies on sedimentation and utilizes settle plates that are exposed to the air for a specific duration. The results are typically reported as colony-forming units (CFU) per plate per unit of time. The passive method has been standardized by the Index of Microbial Air Contamination (IMA), which represents the number of CFUs counted on a Petri dish with a diameter of 9 cm. The dish is left open to the air using the 2/3/3 scheme, which entails a two-hour exposure time, positioning the dish three feet above the floor, and placing it approximately three meters away from walls and major obstacles. The IMA can also be expressed as CFU per square meter (m), square decimetre (dm), or square centimetre (cm) per unit of time[6].

2.2.4 Preparation of Lactophenol Cotton Blue Slide Mounts.

The lactophenol cotton blue (LPCB) wet mount technique is extensively employed for staining and observing fungi and is easily prepared. This preparation consists of three elements: phenol, which eliminates any living organisms; lactic acid, which preserves fungal structures; and cotton blue, which stains the chitin present in fungal cell walls. Place one or two drops of the lactophenol/cotton blue mountant/stain on a microscope slide. The specimen/material immersed in the drop of lactophenol/cotton blue mountant/stain. Holding the coverslip between forefinger and thumb, touch one edge of the drop of mountant with the coverslip edge, and lower gently, avoiding air bubbles, the preparation is now ready for examination under the Microscope [7].

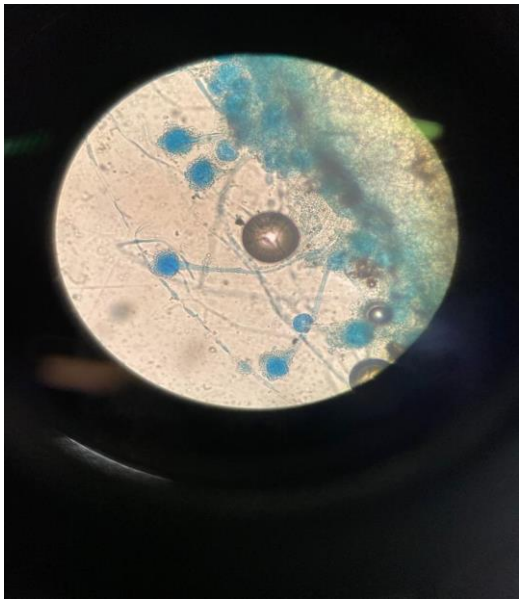

Figure 1: Lactophenol cotton blue Staining

3. Result

3.1 Collection of air samples.

A total 60 no. of air samples (both indoor & outdoor) were collected and evaluated for the presence of different microorganisms on different types of culture media (SDA & PDA with banana peel) which were important for Quality Control in environment. Among these samples, 35 were collected from indoor & 25 from outdoor environments.

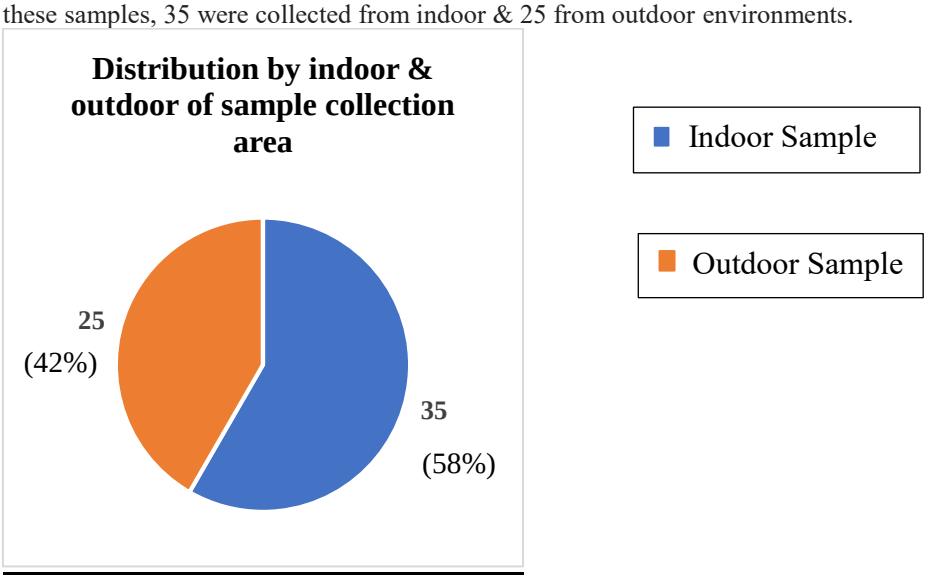

Figure 2: Diagram showing samples collected according to Indoor & Outdoor of sample collection area.

3.2 Number of various fungi isolates.

A variety of fungi have been isolated in this study at 37°C. A total number of 190 fungal isolates has been found in this study. The list of prevalent fungi at 37°C described below in Table-1 & the relevant percentage isolation in pie chart as mentioned in Figure 3.

3.2.1 Table 1: Total number of various fungi isolates from samples.

SL NO.	Type of fungi	No. of isolates
1.	Phoma spp.	29
2.	Rhizopus oryzae	15
3.	Aspergillus niger	66
4.	Penicillium chrysogenum	1
5.	Aspergillus flavus	21
6.	Aspergillus terreus	13
7.	Aspergillus fumigatus	24
8.	Aspergillus parasiticus	1
9.	Acremonium spp.	15
10.	Aspergillus lentulus	1
11.	Aspergillus candidus	1

12.	Rhizomucor pusillus	3

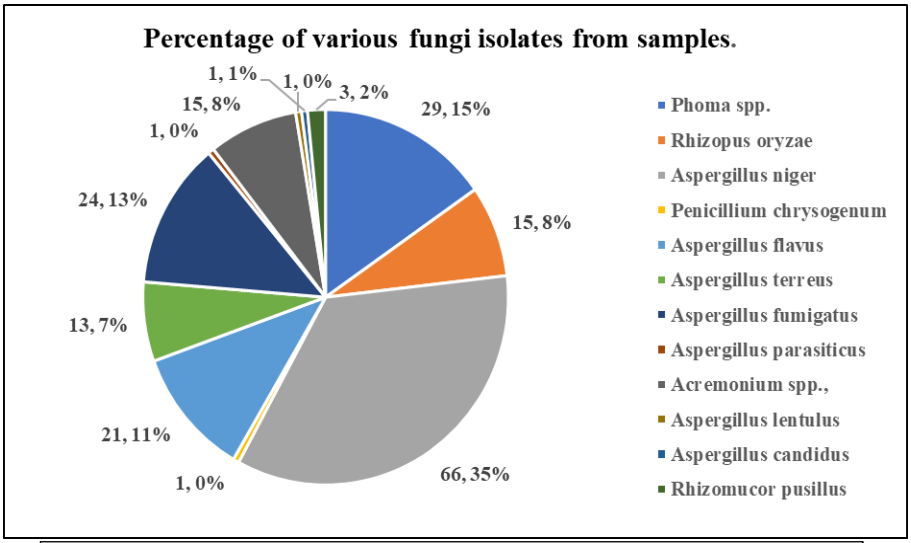

Figure 3: Percentage of various fungi isolates from samples.

Different varieties of fungi isolated in SDA vis-a-vis PDA with BP is depicted in Table 2 and Figure 4. Most notably PDA with Banana Peel Plate helped isolate more fungi than SDA Plate. Phoma spp., Acremonium spp.and Aspergillus niger had substantially grown better in PDA supplemented with BP. However, growth of mucorales was relatively poor in PDA with BP compared to SDA.

3.2.2 Table 2: Number of Fungi in air sample.

SL NO.	Type of fungi	In SDA plate	In PDA with banana peel plate
1.	*Phoma spp.*	12	17
2.	*Rhizopus oryzae*	10	5
3.	*Aspergillus niger*	31	35
4.	*Penicillium chrysogenum*	1	0
5.	*Aspergillus flavus*	12	9
6.	*Aspergillus terreus*	4	9
7.	*Aspergillus fumigatus*	14	10
8.	*Aspergillus parasiticus*	1	0
9.	*Acremonium spp.*	5	10
10.	*Aspergillus lentulus*	1	0
11.	*Aspergillus candidus*	1	0
12.	*Rhizomucor pusillus*	2	1
Total		94	96

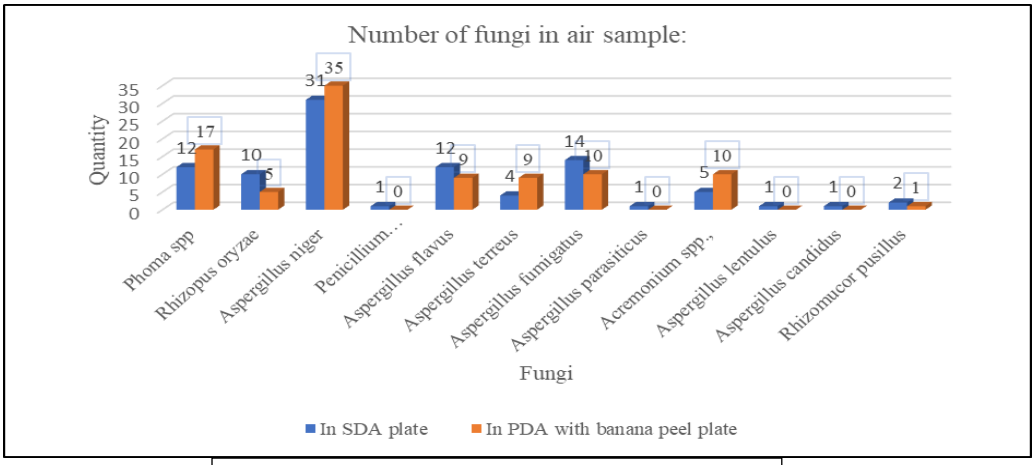

Figure 4: Number of fungi in air sample.

Table no.3 describes the colony morphology of various Fungal genera in PDA with Banana Peel Plate.

3.2.3 Table 3: Colonical Morphology of Different types of Fungi.

SL NO.	Type of fungi	Colonical Morphology
1.	*Phoma spp.*	Flat spreading cottony to velvety & often largely submerged in the medium.
2.	*Rhizopus oryzae*	Whitish/grey fuzzy colonies.
3.	*Aspergillus niger*	Black powdery colony.
4.	*Penicillium chrysogenum*	Blue-green, grey-green, olive-grey colony.
5.	*Aspergillus flavus*	Powdery masses of yellowish green.
6.	*Aspergillus terreus*	Yellowish-brown to dark brown colour.
7.	*Aspergillus fumigatus*	Typically, blue green with a suede-like surface.
8.	*Aspergillus parasiticus*	Dark green colony colour.
9.	*Acremonium spp.*	Colonies are flat, with smooth, wet, velvety or floccose texture, sometimes resembling thin cottony mounds.
10.	*Aspergillus lentulus*	Blue green with a suede-like surface.
11.	*Aspergillus candidus*	White powdery colony.
12.	*Rhizomucor pusillus*	White-green cottony colony.

Figure 5: Colonical morphology of *Rhizomucor pusillus.*

Figure 6: Colonical morphology of *Aspergillus niger* & *Aspergillus fumigatus.*

Figure 7: Colonical morphology of *Phoma spp.*

4. Discussion

At the end of the study, we were able to assess the presence of pathogenic fungi by collecting them in the form of aerosols. Hence these findings were important for Quality Control in environment. The purpose of this study is to define the pathogenic microbial flora of indoor & outdoor air by identifying and comparing the cultivable fungal species. Our research aimed to provide valuable insights into the detection of various microorganisms in aerosolized form within the air. Additionally, we discovered the potential applications of discarded fruit waste, such as mature banana peels, as an innovative agar medium. This medium proved beneficial in cultivating diverse pathogenic microorganisms, facilitating a more comprehensive analysis of our study. The settle-plate technique is commonly employed to investigate the presence of fungi in the air, with Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) being the preferred culture media for this purpose[8]. However, there is an increasing interest in exploring alternative culture media that are readily accessible and cost-effective, such as Banana Peel (BP). In this research, we conducted a comparative analysis to assess the efficiency of SDA and PDA with BP as culture media for the detection and isolation of airborne fungi using the settle-plate method. The findings of our investigation revealed that both these media successfully supported fungal growth from the air samples, although variations were observed in terms of the types and quantities of colonies cultivated on each medium. The settle-plate technique employing SDA and PDA as culture media is widely regarded as the benchmark for monitoring fungal presence in the air due to their exceptional sensitivity and selectivity. However, our findings indicate that PDA supplemented with BP can very well serve as a viable substitute for monitoring fungal presence in the air, particularly in scenarios where SDA is limited in availability or financially impractical. PDA enriched with BP offers an environmentally friendly and easily accessible nutrient source, making it a cost-efficient and sustainable alternative to traditional media[9]. Due to the recent surge in interest in assessing both indoor and outdoor air quality concerning mold, settle plate method using commonly employed media like malt-extract agar (MEA), Sabouraud dextrose agar (SDA), and potato dextrose agar (PDA) with or without antibiotics have become popular. The primary distinction between potato dextrose agar and Sabouraud dextrose agar lies in their compositions. Potato dextrose agar is a culture medium primarily composed of potato infusion, dextrose, and agar. On the other hand, Sabouraud dextrose agar is a culture medium for fungi cultivation, mainly comprising peptone, dextrose, and agar. Potato dextrose agar and Sabouraud dextrose agar represent two distinct types of culture media utilized for cultivating fungi[10]. PDA being deficient in peptones can induce better production of fruiting bodies and structures in fungi, helpful for their identification. In this study, we added Banana Peel with PDA and compared this with SDA by the number & type of growing fungi in both media. The quantity of fungal colonies and the overall fungal diversity were significantly higher on plates containing PDA with Banana Peel compared to SDA plates. These results indicate that PDA with Banana Peel created a more favourable environment for the growth and proliferation of

airborne fungi. The addition of banana peel to PDA further enriches the medium with additional nutrients, vitamins, and minerals, thereby enhancing the growth of microorganisms. By the end of the study, it was evident that a greater number of fungi and microorganisms thrived on PDA with Banana Peel compared to SDA. Banana Peel used in PDA had definitely increased the rate of growth of fungi as evident in this short-term study. However, for long-term studies, researchers should employ this medium to gain further insights into the growth of pathogenic and non-pathogenic fungi and microorganisms.

5. Conclusion

In summary this study revealed the presence of distinct microbial flora in both indoor and outdoor air environments, including species associated with airborne diseases such as *Aspergillus spp*. which are found ubiquitously. We employed various process, such as lactophenol cotton blue mount and slide culture, to identify these fungi. The results demonstrated that PDA with BP fostered higher fungal growth rates and supported a greater diversity of fungal species compared to SDA. This can be attributed to the richer nutrient content present in PDA with BP, which created optimal conditions for fungal growth. While SDA and PDA remain well-established culture media in fungal research, our study highlights the potential value of using BP with PDA as an alternative medium in situations where SDA is not easily accessible or cost prohibitive. It is important to acknowledge the limitations of our study, which focused exclusively on the settle-plate method and did not explore alternative air sampling techniques. Variations in sampling efficiency among different methods may lead to variations in results. Future research could investigate the performance of SDA, PDA with BP in combination with other air sampling methods such as agar strip or impaction samplers. Further exploration is also warranted to assess the potential of combining BP with other media or additives to enhance its nutrient content and expand the range of fungal species it can support. Modifying the composition of BP in agar may offer an opportunity to optimize its nutrient content and improve its effectiveness as a culture medium for airborne fungi.

In conclusion, the comparative analysis of SDA, PDA, and BP in studying fungi in the air using the settle-plate method revealed that SDA and PDA were more effective in terms of fungal growth and diversity. However, the use of BP as an alternative media has potential, especially in situations where cost-effectiveness and sustainability are paramount. Overall, PDA with BP offers a more nutrient-rich, cost-effective, and sustainable alternative to SDA for the cultivation of airborne fungi. It also provides a more versatile medium for the study of airborne microorganisms, including fungi. Therefore, we recommend the use of PDA with BP for the cultivation and enumeration of airborne fungi in future studies.

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