

Automated Exposure of Colorimetric Fluctuations in *Aspergillus Flavus* to Permit Feasible Extraction of Antibiotics

Pradeep Nazareth

Alva's Institute of Engineering and Technology,
Moodabidre
Karnataka, India
pradeep.nitk2017@gmail.com

Sannidhan M S

NMAM Institute of Technology
(Nitte Deemed to be University)
Karnataka, India
sannidhan @nitte.edu.in

Jason Elroy Martis

NMAM Institute of Technology
(Nitte Deemed to be University)
Karnataka, India
Jason1987martis @nitte.edu.in

Pratheeksha Hegde N

NMAM Institute of Technology
(Nitte Deemed to be University)
Karnataka, India
pratheeksha.hegde@nitte.edu.in

Abstract- This research article focuses on the detection of the mature stage of *Aspergillus flavus* fungus using an automated approach. *Aspergillus flavus* is a pathogenic fungus known for causing diseases in crops and producing carcinogenic mycotoxins. Detecting the mature stage is crucial for optimizing the production of antibiotics such as Aspergillic acid, which is abundantly produced during this phase. Manual color detection methods are labour-intensive and prone to errors. Existing techniques face challenges due to lighting conditions and background issues. To overcome these limitations, we propose a two-part automated process that involves background elimination using comparative thresholding techniques and mapping the mature phase on the Lab* scale. This approach eliminates lighting conditions and enhances accuracy in identifying the mature phase. By automating the detection process, our method offers a significant advancement in the production of antibiotics and reduces the reliance on manual intervention. The recommended methodology has the competence to upgrade efficiency in the pharmaceutical industry and educational research on fungal growth.

Keywords-- *Aspergillus flavus*, Fungal growth, Automated detection, Mature stage, Antibiotic production.

I. INTRODUCTION

Mycology is a biological field that studies mycota, also designated as fungi. Mycota appear with distinct shapes and sizes, that can also impact humans in various ways. Some of them can be used for making fire, medicine, food, or drugs, while others can be harmful or poisonous. Also, they can be a source of danger and dangers, such as toxicity or infections. An example of a pathogenic organism is *Aspergillus flavus*, which is a pretty non-aggressive devious pathogen disturbing various crop. It induces diseases in different crops [1]. Numerous pathogenic fungi affect both animals and plants. Certain types of these accumulate on plants and through consumption of the same infect humans. In minor circumstances, fungi can also be transferred indirectly to humans. For instance, when domestic animals consume poisoned grain, further products derived from them may inhibit unpredictable levels of fungal toxins [2].

Contaminants produced by *Aspergillus flavus* has a huge impact and can supper deeply and extensive, and even individuals who have certainly not contracted the mould can be affected. Research has additionally confirmed that, apart from fumigatus, flavus is the next most ubiquitous species uncovered in human infections. It typically appears in soil,

corpse vegetation, and animals. Primarily, Flavus colonies stage a yellow-green emergence, which progressively shifts to a darker shade of green color across time. The texture is reminiscent of wool or cotton and occasionally granular [3,4].

Aspergillic acid is an organic acid produced by all species of aspergillus. It is used heavily in the production of antibiotics such as penicillin [5,6]. Though antibiotics can easily be produced using this acid, the difficulty lies in extracting the ethanoic acid to its purest form. This is a hassle since the fungus produces this acid in minimal quantities during its final stages. Several studies have revealed that all the species of this fungus *Aspergillus flavus* produces large quantities of Aspergillic acid during its maturity phase.

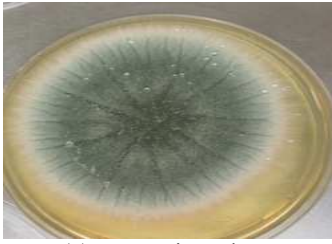
Flavus reveals allergenic traits and is also a recognized pathogen affecting plants, humans, and animals. It can further lead to aspergillosis leading to the derivation of Aflatoxin, known to be a carcinogenic mycotoxin. Moreover, there have been reports mentioning the impact of flavus in nasal sinus lesions and other diseases of invasive nature [7]. This mold is capable of thriving within a expansive range of temperatures and pH levels. The most likely growth rates are at 33°C, although the survival of the same demands the range of temperatures spanning from 10-48 degree Celsius. Similar to other molds, Flavus also demands moist environment for development. Additionally, it has the survival capacity even in the areas with minimum water activities, indicating lowest value of 0.78. Even it demonstrates noteworthy pH forbearance, exhibiting the power to grow in settings of diverse pH values. It can survive in lowest possible pH of 2.1 and also a highest value of 11.2. However, the most favorable development is observed in a range of pH between 3.4 to 10 [8]. The growth of the species is categorized into three different stages which is portrayed in Fig. 1.



(a) Initial Phase



(b) Mature Phase



(c) Extraction Phase

Fig. 1. Phases of *Aspergillus flavus*' growth

Though the fungus has pathogenic abilities, it also has some advantages. When *Aspergillus flavus* reaches maturity, it creates a coat of hydrophobic proteins that repel water on contact keeping the surface dry. Extraction of the Aspergillitic acid is performed when the fungus reaches maturity. Fig 1(c) clearly shows the fungus reaching the maturity stage, sending off a light greenish tinge embedded on a white seabed. During this phase the fungus produces the highest amount of Aspergillitic acid. Achieving the judgement of colour change in this phase using manual labour is too tiresome. It can cause errors to even the most expert mycologists, thereby causing issues in the extraction process. In the current situation, the manual practice of colour detection requires a lot of human resources. Hence, automated colour detection in fungi plays an essential role in the education field and industries to study fungal growth. Generally, it is common for fungi to change colour and even texture under different culture conditions like pH, culture medium and temperature [9].

In this exertion, we extend the proposal of an automated methodology to aid the detection of mature stage of *aspergillus flavus* fungus. Though existing techniques exist that can detect the color of the fungus they fail terribly because of lighting conditions, background issues etc. We overcome this by inducing a two-part process which eliminates the background from the fungus using comparative thresholding techniques [10]. We also identify the last stage of the fungus by eliminating all lighting conditions and mapping the mature phase on the Lab* scale [11]. This twofold process helps the mycologists to decide the accurate mature phase of the fungus without manual intervention thereby increasing the antibiotic production process.

II. LITERATURE SURVEY

Image analysis techniques have proven invaluable in multiple domains, including agriculture and medical diagnostics. Teena et al. [12] conducted research on the detection of fungal contamination in different date varieties using RGB color imaging. Their study involved categorizing samples into three groups: untreated control, surface sterilized and rinsed, and surface sterilized, rinsed, and fungal inoculated. By employing an RGB color imaging system, the authors captured color images of the date samples and utilized classification models to assess the accuracy of detecting fungal infection. The results demonstrated the method's effectiveness, with the Fard variety achieving the highest accuracy (97%) in the two-class model, followed by Khalas (100%) and Naghal (99%). This research sheds light on the potential of RGB color imaging as a reliable tool for identifying fungal contamination in agricultural contexts.

The detection of tuberculosis (TB) in chest X-rays is a critical area of research in medical imaging. Reference [13] provides a comprehensive review of recent advancements in computer-aided TB detection. The authors emphasize the significance of contrast enhancement techniques in improving the visibility of lung boundaries and tissue surfaces in chest X-ray images. Various methods, such as histogram analysis, histogram equalization (HE), wavelet-based transformations, and piecewise linear models, are explored to enhance the effectiveness of TB detection algorithms. The review also discusses an energy normalization technique that shows promising results in improving lung segmentation across diverse X-ray datasets. This literature review offers valuable insights into the modern state-of-the-art techniques in TB detection and underscores the potential for further advancements in this important field.

Moving forward, other studies have explored image analysis techniques in different domains. Camargo et al. [14] propose an a method relying on image processing techniques for discovering diseases of plants in the form of visible symptoms. Their approach involves transforming RGB images into specialized color spaces and employing histogram analysis for segmentation. Barbedo [15] presents a method for distinguishing healthy and infested maize plants using digital images, achieving remarkable accuracy. Pagola et al. [16] propose a novel method for quantifying nitrogen deficiency in barley leaves using color channel manipulation and principal component analysis. Patel et al. [17] explore HE techniques for contrast enhancement in image processing. Kong [18] highlights the importance of global HE and discusses alternative HE methods. Padmavathi and Thangadurai [19] emphasize the significance of digital image processing in medical and biological sciences. Kanan and Cottrell [20] challenge the assumption that color-to-grayscale conversion has minimal impact on image recognition, presenting a comparative analysis of grayscale algorithms.

In conclusion to an extensive literature review, it becomes evident that color changes in plants serve as a valuable indicator for growth rate detection. This conclusion is supported by a thorough examination of articles pertaining to color image processing techniques. Among these techniques, HE emerges as a particularly effective method for identifying and aligning color components. Its application in plant analysis shows promise for advancing our understanding of growth patterns.

III. PROPOSED SYSTEM

The research framework we propose encompasses a comprehensive system comprising five key phases as depicted in Fig. 2.: Image Acquisition, Pre-processing, Channel Conversion, Background (BG) Removal, and Class Mapping. Each phase plays a crucial role in the overall process, contributing to the accurate analysis of the acquired images. In the ensuing sections, we organise a detailed overview of the methodology employed in each phase, highlighting the specific steps involved and their significance in achieving reliable results. By following this systematic approach, we aim to enhance the effectiveness of image analysis and facilitate a deeper understanding of the underlying patterns and characteristics of data.

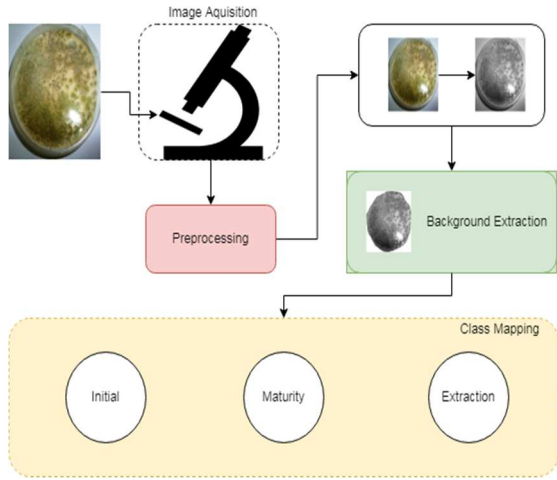


Fig. 2. The Proposed Research Framework for Image Categorization.

A. Image Acquisition

During the initial stage of our research, we captured the fungal samples using a standard camera setup integrated with a microscope. This setup provides optimal conditions for acquiring high-quality images of fungal specimens. The samples are carefully placed on a flat, transparent surface within the microscope's field of view. To ensure consistent lighting conditions and minimize BG interference, a constant light source is employed, illuminating the samples against a white BG [21]. This controlled setup allows for precise and accurate image acquisition, facilitating subsequent processing and analysis tasks. By capturing the fungal samples under these controlled conditions, we aim to mitigate any potential variations and artifacts that could affect the accuracy of our research findings.

B. Image pre-processing

This stage enhances the effectiveness of maturity prediction by enabling efficient decision-making capabilities. To ensure optimal extraction of fungal features from images, it is essential to appropriately handle image size and BG noise attributes, taking into account the specific domain [22]. The following sub-techniques are integrated to efficiently extract image features.

- **Image resizing :** This phase resizes the input image to the size 200 by 200 pixels.
- **BG Noise Removal:** The RGB color model undergoes processing to eliminate BG noise. Initially, the image is converted to grayscale, resulting in the creation of an image in grayscale form. This form of image is subsequently prepared in binary format and employed for the Gaussian-based removal of background noise from the image [22]. The mathematical explanation of this process is provided by equations (1) and (2).

$$Img_{(x,y)}^G = (.2989 * Img_{(x,y)}^R) + (.587 * Img_{(x,y)}^G) + (.114 * Img_{(x,y)}^B) \quad (1)$$

$$Img^{Gauss} = (2\pi\sigma^2)^{-1} * e^{-\frac{(x^2+y^2)}{2\sigma^2}} \quad (2)$$

In equation (1), $Img_{(x,y)}^G, Img_{(x,y)}^R, Img_{(x,y)}^G$ and $Img_{(x,y)}^B$ represents the grayscale, red, green and blue channels, respectively. In equation (2) Img^{Gauss} represents the gaussian image noise reduction with a σ^2 a factor of 0.6.

- **Grayscale conversion:** Grayscale conversion of a color image is accomplished in this section by computing mean values as per the formulation presented in equation 1. Fig. 3. illustrates the grayscale representation of the fungal sample [23].



Fig. 3. (a): Original RGB image (b): Converted gray scaled image

C. Channel conversion

In this section, we delve into the process of channel conversion, a vital aspect of color image processing. Fig. 4. illustrates a significant transformation as the RGB image undergoes conversion to highlight the blue color component, serving the purpose of extraction. By reducing the values of the channels corresponding to red and blue to zero, the intensity of the channel representing green color is effectively amplified, resulting in a striking and vibrant representation. Fig. 5. exemplifies this conversion process by showcasing the resized fungal image converted into a greener color component while disregarding the red and bluish components. This deliberate channel conversion enables a focused analysis, revealing the desired visual information with clarity and precision.

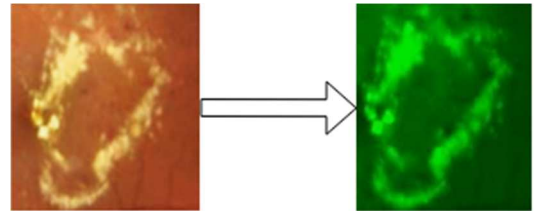


Fig. 4. Visual presentation of channel conversion

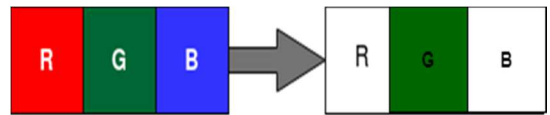


Fig. 5. RGB value of each pixel in channel conversion

D. BG Removal

One crucial stage in the BG removal process involves generating a binary mask known as the Otsu mask. To efficiently remove BG information, we employ Nobuyuki Otsu's binary mask technique. This method utilizes a histogram-based approach to evaluate edge criteria by calculating the intra or inter-class variance. The selection of this criterion aims to minimize variances by assigning weight probabilities to the variances between classes. The computation of this aspect is represented by Equations (3)-(5).

$$W_0 = \frac{\sum_{i=0}^{n-1} ip(i)}{\omega_0(t)}$$

$$W_1 = \frac{\sum_{i=0}^{n-1} ip(i)}{\omega_1(t)}$$

$$\mu_T = \sum_{i=0}^{L-1} ip(i)$$

W_0 and W_1 symbolize the probabilities allotted to two classes divided by a threshold value, T. The class probability $W_{(0,1)}(T)$ is derived by inspecting different bins of the histogram. Subsequently, the product of pre-processed RGB image fed as an input and the computed mask is obtained, enabling the extraction of fungal sample regions.

It is equally important to make a clear note that in the resulting image, pixels belonging to the original image will preserve their values only if the same pixel in the binary masked image appears to be one. Contrarywise, if the same pixel in the binary masked image appears to be zero, the resultant image will adopt the binary mask's pixel value. For a visual representation of the binarization process, refer to Fig. 6. Additionally, Fig. 7 illustrates the pixel-wise outcome of binary mask's application with a value of one for all color components, while Fig. 8 displays the consequence of binary mask's application with a value of zero for all color components.



Fig. 6. Visual representation Binary Masking

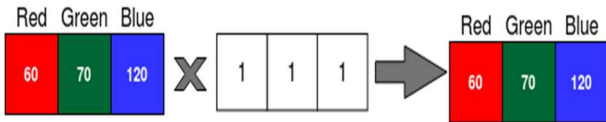


Fig. 7. Pixelwise RGB value when mask is 1.



Fig. 8. Pixelwise RGB value when mask is 0.

E. Class Mapping

Class mapping is a very crucial stage of the proposed system which maps the input image to the stage of maturity based on the calculation of threshold value achieved. To estimate the threshold value, Lab* color transformation technique is used. The process of transformation is elegantly expressed through the mathematical representation provided in equations (6) to (9). These equations precisely delineate how the transformation takes place, allowing for a comprehensive understanding of the underlying mechanisms involved.

$$(3) \quad L^* = 116 * fun\left[\frac{Y}{Y_n}\right] - 16 \quad (6)$$

$$(4) \quad a^* = -500 * \left(fun\left[\frac{Y}{Y_n}\right] - fun\left[\frac{X}{X_n}\right] \right) \quad (7)$$

$$(5) \quad b^* = -200 * \left(fun\left(\frac{Z}{Z_n}\right) - fun\left(\frac{Y}{Y_n}\right) \right) \quad (8)$$

$$fun(y) = \begin{cases} y^{\frac{1}{3}}, & \text{if } y > \delta^3 \\ \frac{8}{58} + \frac{y}{3\delta^2}, & \text{else} \end{cases} \quad (9)$$

In this context, the symbol δ represents a specific constant, precisely $\delta = 12/58$. The terms X_n , Y_n and Z_n are CIE XYZ tri stimulus amounts of the associated white point. To shed light on the illuminant D65, which has a normalization value of $Y=100$, we delve into the distinctive details of the variables X_n , Y_n and Z_n , which take on the respective values: 095.047, 0100.000 and 0108.883.

To avoid an infinite slope at $t=0$, we split the distribution function 'F' into two distinct parts over its domain. Under certain conditions, 'F' exhibits cubic behavior, specifically below a value of $t=y$, and matches the value $y^{\frac{1}{3}}$ at $t=0$, including both the value and slope. In our unique approach to fungal analysis, we employ a^* estimated values from the fungal sample. These values are vital for making crucial decisions about antibiotic extraction.

Upon accomplishing background image processing, all the a^* values are meticulously gathered and summed. This total is then used to calculate the mean a^* value. By analyzing these values, we can effectively categorize the fungus's maturity phase into three distinct stages: Initial, Mature, and Extraction. Through extensive experimentation, we have successfully determined threshold values for the three defined maturity categories based on equations (6), (7), and (8) when applied to a large number of collected samples. For reference, the specific threshold values for the defined lemon categories are detailed in Table I, serving as a useful guide in our research.

TABLE I. THRESHOLD a^* VALUES

Category	Percentage of a^*	a^* threshold values
Initial	10 to 30	Less than or equal to -8
Mature	30 to 60	Greater than -8 and less than or equal to -3
Extraction	60 to 100	Greater than -3 and less than or equal to 0

IV. RESULTS & DISCUSSION

In this segment, we present the empirical findings derived from the application of our novel methodology, unveiling the remarkable results achieved through our carefully conducted experiments.

A. Dataset Collection

The collection phase involved conducting a comprehensive analysis on 150 samples of *aspergillus flavus* fungus,

encompassing various categories and maturity levels. These samples were carefully cultivated under controlled laboratory conditions to ensure ideal growth conditions. To establish a reliable baseline, around fifty fungal samples were obtained from experienced microbiologists specializing in mycology, each representing three distinct maturity levels.

B. Experimental Observations

In this section of the experiment, a thorough evaluation was conducted on a diverse set of samples from different categories of *Aspergillus Flavus*. Approximately fifty samples were obtained for each category, and the a^* threshold values were extracted as outlined in Table I. These threshold values play a crucial role in the analysis. Afterward, we meticulously compared the experimental results obtained from our research with the meticulously crafted theoretical descriptions of the various fungus categories. This rigorous analysis allowed us to draw meaningful conclusions and gain valuable insights into the accuracy and effectiveness of our proposed methodology. The findings are presented in Table II, providing a comprehensive overview that facilitates a meaningful comparison between the observed characteristics and the expected attributes of each fungus category.

TABLE II EXTRACTED a^* THRESHOLDS FOR ASPERGILLUS FLAVUS CATEGORIES.

Stage of Maturity	Number of Samples Under Test	Number of Samples Cleared the Test	Rate of Pass (%)
Extraction	50	45	90
Maturity	50	47	94
Initial	50	46	92

C. Graphical analysis

To conduct a comprehensive analysis of the test results, we generated a graph based on the data provided in Table II. This graph displays the number of samples on the x-axis and the corresponding calculated a^* values on the y-axis. The samples that successfully passed the test fall within the designated range for a specific maturity level, which is detailed in Table 1. However, when a sample fails the test, its a^* value falls outside this range. For a visual representation, please refer to Figures 9 – 11, where we present the graphical analysis of these findings. These visualizations offer unique insights into the performance and distribution of the tested samples, reinforcing the reliability and validity of our methodology.

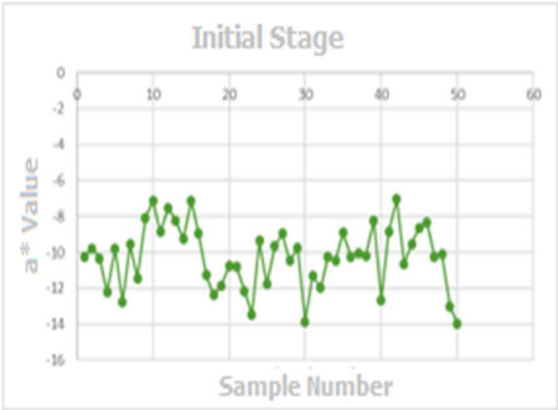


Fig. 9. Initial Stage - Graph of a^* values

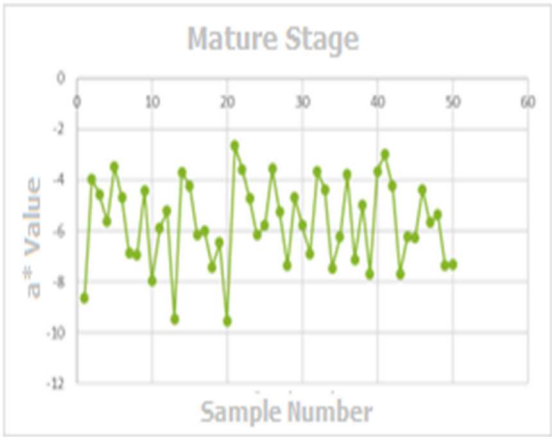


Fig. 10. Mature stage - Graph of a^* values.

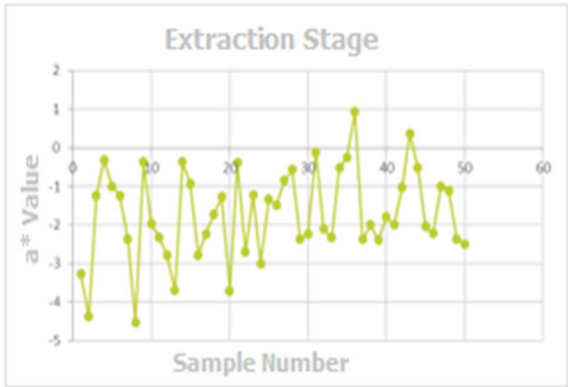


Fig. 11. Extraction stage - Graph of a^* values.

Fig. 11 provides conclusive evidence showcasing the certainty of a^* values obtained during the extraction phase, which range from -3 to 0. This robust range of values demonstrates the system's effectiveness in accurately identifying the fungus, making it a feasible solution for antibiotic extraction purposes.

D. Comparative assessment with traditional approaches

In this section, we meticulously conducted an extensive comparative evaluation, scrutinizing the performance of our innovative color image processing techniques in direct comparison to long-established conventional methodologies. The outcomes of this in-depth comparative analysis are comprehensively presented in Table III, offering a detailed insight into the experimental observations.

TABLE III COMPARATIVE EVALUATION OF PROPOSED TECHNIQUE WITH THE TRADITIONAL APPROACHES.

Technique	Average efficiency (%)
CIELUV	51.28
CIELAB	74.66
HCL	83.17
Proposed	92

Based on the data depicted in the table, it is evident that the proposed method outperforms all conventional approaches, thereby establishing the value of its implementation.

V. CONCLUSION

In this research exertion, we have achieved successful detection of the extraction phase of *Aspergillus Flavus*, which represents a critical task in our proposed work. By implementing a computer-based system, we have not only provided a cost-effective preliminary approach to detecting color changes but also achieved improved performance ratios. To enhance the system's value-added services, we have incorporated an automated notification module that specifically notifies end-users revealing the extraction stage. This integration has significantly reduced the manual effort required for sample collection and detection processes.

One notable limitation of the system is its sensitivity to minor variations in green channel values, which can lead to inaccuracies in identifying the precise growth stage. To address this drawback, future improvements can be made by incorporating a neural network learning technique. By training the system to recognize even minute changes in the green channel, it has the potential to achieve enhanced accuracy and performance. This approach would enable the system to accurately identify and classify a greater number of stages in fungal growth, thereby improving its overall capabilities.

Future work can focus on refining the color detection algorithm to address the system's sensitivity to minor green channel variations. By integrating neural networks, we can significantly enhance the system's accuracy and broaden its ability to effectively classify a diverse spectrum of *Aspergillus Flavus* growth stages. The incorporation of such cutting-edge technologies empowers the system to make precise and comprehensive assessments, contributing to a more robust and reliable analysis of the fungus's developmental phases. By broadening the scope of value-added services, rigorously validating on an extensive dataset, and fostering collaborations with domain experts, we can achieve remarkable enhancements in the system's overall performance and practicality. These strategic steps ensure the system's ability to address a diverse array of challenges and cater to a broader range of applications, making it a valuable asset in various domains.

REFERENCES

- [1] Bhatnagar, D., Ehrlich, K. C., Moore, G. G., & Payne, G. A. (2014). *Aspergillus Aspergillus flavus*. Encyclopedia of food microbiology, 1, 83-91. doi:10.1016/B978-0-12-384730-0.00012-4
- [2] Sannidhan, M. S., Martis, J. E., Nayak, R. S., Aithal, S. K., & Sudeepa, K. B. (2023). Detection of Antibiotic Constituent in *Aspergillus flavus* Using Quantum Convolutional Neural Network. International Journal of E-Health and Medical Communications (IJEHMC), 14(1), 1-26.
- [3] Martis, J. E., Sudeepa, K. B., Sannidhan, M. S., & Bhandary, A. (2020, August). A rapid automated process for organizing bacterial cluster segments using deep neural networks. In *2020 Third International Conference on Smart Systems and Inventive Technology (ICSSIT)* (pp. 963-968). IEEE.
- [4] Samanta, A., Saha, A., Satapathy, S. C., Fernandes, S. L., & Zhang, Y. D. (2020). Automated detection of diabetic retinopathy using convolutional neural networks on a small dataset. Pattern Recognition Letters, 135, 293-298. doi:10.1016/j.patrec.2020.04.026
- [5] Wang, F., Wei, M., Duan, X., Liu, X., Yao, S., Wang, J., Zhu, H., Chen, C., Gu, L., & Zhang, Y. (2020). Identification, synthesis and biological evaluation of pyrazine ring compounds from *Talaromyces minioluteus* (*Penicillium minioluteum*). Organic Chemistry Frontiers : An International Journal of Organic Chemistry / Royal Society of Chemistry, 7(22), 3616-3624. doi:10.1039/D0QO01030H
- [6] Chişca, D., Sterici, V., & Croitor, L. (2016). Mixed-ligand binuclear, 1D and 2D polymeric compounds of Cu (II), Zn (II) and Cd (II) with pyrazine carboxamide. In *Materials Science and Condensed Matter Physics* (pp. 135-135). Academic Press.
- [7] Hedayati, M. T., Pasqualotto, A. C., Warn, P. A., Bowyer, P., & Denning, D. W. (2007). *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. *Microbiology*, 153(6), 1677-1692.
- [8] Sugita, C., Makimura, K., Uchida, K., Yamaguchi, H., & Nagai, A. (2004). PCR identification system for the genus *Aspergillus* and three major pathogenic species: *Aspergillus fumigatus* *Aspergillus flavus* and *Aspergillus niger*. *Medical Mycology*, 42(5), 433-437.
- [9] Sehgal, I. S., Choudhary, H., Dhooria, S., Aggarwal, A. N., Bansal, S., Garg, M., Behera, D., Chakrabarti, A., & Agarwal, R. (2019). Prevalence of sensitization to *Aspergillus flavus* in patients with allergic bronchopulmonary aspergillosis. *Medical Mycology*, 57(3), 270-276. doi:10.1093/mmy/myy012 PMID:29566248
- [10] Sannidhan, M. S., & Bhandary, A. (2018, December). Lemon Maturity Estimator: An Approach Using Color Image Processing Techniques. In *2018 International Conference on Electrical, Electronics, Communication, Computer, and Optimization Techniques (ICEECOT)* (pp. 1213-1218). IEEE.
- [11] Barbu, T., Ciobanu, A., & Mihaela, C. (2012, June). Automatic color-based image recognition technique using LAB features and a robust unsupervised clustering algorithm. In *Proceedings of the 13th WSEAS international conference on automation and information (ICAI '12), Iasi, Romania* (pp. 13-15).
- [12] Teena, M., Manickavasagan, A., Alsadi, A., Alyahyai, R., Deadman, M., & Alismaili, A. (2016). RGB colour imaging to detect *Aspergillus flavus* infection in dates. *Emirates Journal of Food and Agriculture*, 28(10), 683. doi: 10.9755/ejfa.2016-01-070
- [13] Hina, K., Khalid, S., & Akbar, M. U. (2016). A review on automatic tuberculosis screening using chest radiographs. 2016 Sixth International Conference on Innovative Computing Technology (INTECH). doi: 10.1109/intech.2016.7845039
- [14] Camargo, A., & Smith, J. (2009). An image-processing based algorithm to automatically identify plant disease visual symptoms. *Biosystems Engineering*, 102(1), 9-21. doi: 10.1016/j.biosystemseng.2008.09.030
- [15] Barbedo, J. G. A. (2013). Digital image processing techniques for detecting, quantifying and classifying plant diseases. SpringerPlus, 2(1). doi: 10.1186/2193-1801-2-660
- [16] Patel, O., Maravi, Y. P. S., & Sharma, S. (2013). A Comparative Study of Histogram Equalization Based Image Enhancement Techniques for Brightness Preservation and Contrast Enhancement. *Signal & Image Processing : An International Journal*, 4(5), 11-25. doi: 10.5121/sipij.2013.4502
- [17] Kong, N. (2013). A Literature Review on Histogram Equalization and Its Variations for Digital Image Enhancement. *International Journal of Innovation, Management and Technology*. doi: 10.7763/ijimt.2013.v4.426
- [18] Patel, O., Maravi, Y. P. S., & Sharma, S. (2013). A Comparative Study of Histogram Equalization Based Image Enhancement Techniques for Brightness Preservation and Contrast Enhancement. *Signal & Image Processing : An International Journal*, 4(5), 11-25. doi: 10.5121/sipij.2013.4502
- [19] Kanan, C., & Cottrell, G. W. (2012). Colour-to-Grayscale: Does the Method Matter in Image Recognition? PLoS ONE, 7(1). doi: 10.1371/journal.pone.0029740
- [20] Kanan, C., & Cottrell, G. W. (2012). Colour-to-grayscale: does the method matter in image recognition. PloS one, 7(1), e29740.
- [21] Martis, J. E., Sannidhan, M. S., Aravinda, C. V., & Balasubramani, R. (2023). Car damage assessment recommendation system using neural networks. *Materials Today: Proceedings*.
- [22] Sannidhan, M. S., & Chaitra, K. M. (2019, August). Assessment of image enhancement procedures for matching sketches to photos. In *2019 IEEE International Conference on Distributed Computing, VLSI, Electrical Circuits and Robotics (DISCOVER)* (pp. 1-5). IEEE.
- [23] Sannidhan, M. S., Martis, J. E., Aravinda, C. V., & Bhatnagar, R. (2023, March). Correction of an Image for Colour Blindness Using the Fusion of Ishihara Filter and Histogram Equalization. In *The International Conference on Artificial Intelligence and Computer Vision* (pp. 294-304). Cham: Springer Nature Switzerland.