WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Validation of the ALERT™ Plant Pathogen Test Kit and Implementation of an Arduino-Based Real Time Monitoring System in a Cacao (*Theobroma cacao* L.) Plantation.

April Rose O. Melana[1], Cyrus Chin G. Labos[1], Mikaellah R. Pama, Adin Marvin B. Azarraga[2] and Lilia M. Fernando-Corpuz[2]

[1]Daniel R. Aguinaldo National High School, Matina, Davao City, Philippines
[2]Institute of Crop Science, University of the Philippines Los Baños, College, Laguna 4031Philippines

ABSTRACT

Objectives. The study aims to create a device that monitors the presence of *Aspergillus flavus* by tracking environmental temperature as a primary factor for fungal growth. It also aimed to validate the effectiveness of the ALERT Plant Pathogen Test Kit for pathogen detection in cacao (*Theobroma cacao* L.) plantations, focusing on a rapid, accurate, and easy method for non-laboratory needs.

Methods. Cacao beans were fermented at room temperature and categorized into three variables—healthy, infected, and naturally healthy or infected. These beans were then subjected to real-time monitoring using the ALERT device. Upon detection of the potential presence of A. *flavus*, the cacao beans underwent pathogen detection via the ALERT Plant Pathogen Test Kit, encompassing three (3) main steps: triage screening, DNA extraction, and colorimetric sensing. Following the acquisition of results, the remaining samples were submitted to an external laboratory for further polymerase chain reaction (PCR) testing to validate the findings.

Results. The detection of A. *flavus* in cacao bean samples was initially done using a monitoring system device, followed by validation through the ALERT Test kit. The results were further confirmed through PCR testing, proving the reliability of the detection process. The alignment between visual assessments and PCR results is significant, enhancing the accuracy of visual inspection and confirming the reliability of the PCR assay. The ALERT device's consistency suggests its potential as a cost-effective, rapid, and user-friendly method for pathogen detection.

Conclusion. The study confirmed the ALERT Kit's effectiveness in confirming suspected A. *flavus* and using a real-time monitoring system for fermenting cacao beans A. *flavus*, confirming the kit's accuracy.

Keywords: *ALERT Kit, cacao, A. flavu*

INTRODUCTION

The Philippines has cacao (*Theobroma cacao* L.) production, with the Davao Region accounting for 80.6% of the country's total cacao production in 2021. The crop is highly regarded for its top-quality beans, which are utilized to make a range of chocolate products (Medenilla, 2022). *Aspergillus flavus*, a mycotoxin-producing fungus, poses significant challenges in post-harvest cacao management, particularly affecting cacao beans. These fungi can contaminate cacao during storage and processing, producing harmful mycotoxins like aflatoxins, which are detrimental to human health and food safety (Chandra et al., 2021). Traditional methods for detecting pathogens, such as PCR, ELISA, and LAMP, are often hindered by the need for specialized equipment and trained personnel. This complexity can restrict their use in field settings, limiting their practical application (Franco et al., 2019).

Biosensors are advanced analytical tools that combine a biorecognition element with an electrochemical transducer. This technology offers rapid detection, simplicity of use, cost-effectiveness, and high levels of sensitivity (Naresh & Lee, 2021). A team of professional scientists from the University of the Philippines Los Baños (UPLB), under the leadership of Dr. Lilia M. Fernando-Corpuz, PhD, developed a nanobiosensor for the DNA-based detection of the A. *flavus*. It provides rapid and accurate detection of the aforementioned phytopathogen. However, the kit was only used for clinical testing. For this reason, the researchers plan to validate the pathogen detection kit in the field.

The environmental temperature and relative humidity play a significant role in the growth of *Aspergillus flavus*. The A. *flavus* grows best at high temperatures (37°C) and high humidity (84%). These factors must be monitored to ensure the beans are safe from contamination and fungal metabolism. Before the detection, the researchers propose to create a device that monitors the current state of the environment to track the presence of A. *flavus* through the detection of its thriving conditions in a cacao tree.

MATERIALS AND METHODS

This section outlined the materials and methods used in the study. It includes the collection of all materials, both electronic and experimental, the device's coding, the assembling of the device, along with the detailed steps for the ALERT Kit, and the compliance of PCR testing. These procedures ensured the reliability and accuracy of the research.

Collection and Preparation of Materials

Collection of Robotic Materials

Dr. Lilia M. Fernando, PhD, from the University of the Philippines Los Baños in Laguna, provides the ALERT Plant Pathogen Test Kit. The Arduino Uno R3, AA batteries, jumper wires, breadboard, DHT11, SSD1306 1280x32 display, and active buzzer were all purchased from an electronic parts supplier, namely CreateLabz, located in the EC Business Center, C.M. Recto St., beside Avida Towers, Davao City, Davao Del Sur.

Collection of Cacao Beans

All cacao beans used for this experiment were obtained on a farm in Mintal, Davao City, Davao Del Sur. Authenticity was certified by the City Agriculturist Office, located at Pichon St., Davao City (Appendix A).

Collection of A. *flavus and primers*

All probes of Aspergillus flavus were used for PCR testing, and the ALERT Kit was given to us by Dr. Lilia M. Fernando, PhD. The certification of the A. *flavus* probes was done by Dr. Lilia M. Fernando, PhD, from UPLB, located at Los Baños, Laguna (Appendix B).

The primers needed for the PCR testing were bought at DIAMED, a laboratory enterprise in Los Baños, Philippines. Its authenticity was verified through the invoice sent by DIAMED (Appendix C).

Coding of the device

The researchers used the C++ language in the Arduino IDE application to code. The code used is from the Arduino Get Started website, modified by the researchers to meet the standards of the study (Appendix D).

Table 1. *References for the A. flavus' Assigned Threshold Level*

References (within Southeast Asian countries)	Approximated Favorable Temperature for A. *flavus*	Approximated Favorable Humidity for A. *flavus*
Indonesia: (Prasetia et al., 2024), (Al-zaban et al., 2023)	37°C =>	83% =>
Thailand: (Jaibangyang et al., 2021)	35°C =>	80% =>
Philippines: (FAO-WHO, n.d.)	40°C =>	90% =>
Average:	> 37°C	> 84% =>

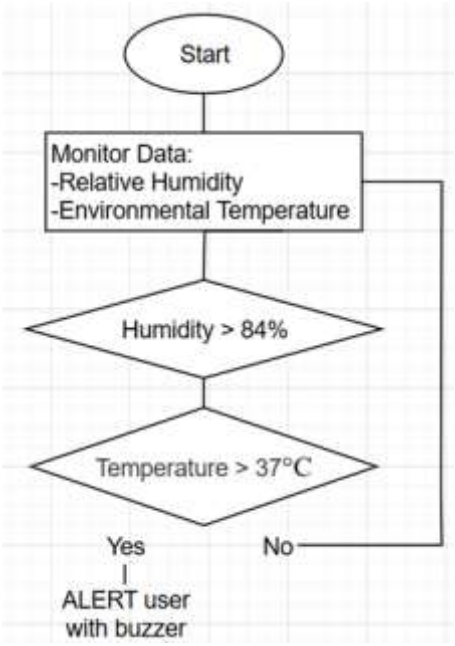


Figure 1. *Program Flowchart of the Monitoring System*

Assembling of the Device

The final monitoring device shown in Figure 4 was assembled following the wiring diagram illustrated in Figure 3.

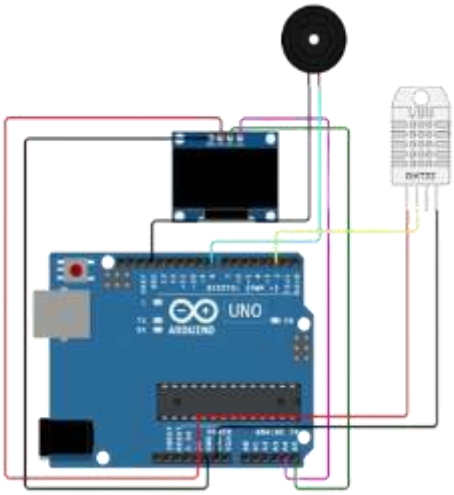


Figure 3. *Wiring Diagram of the Monitoring Device*

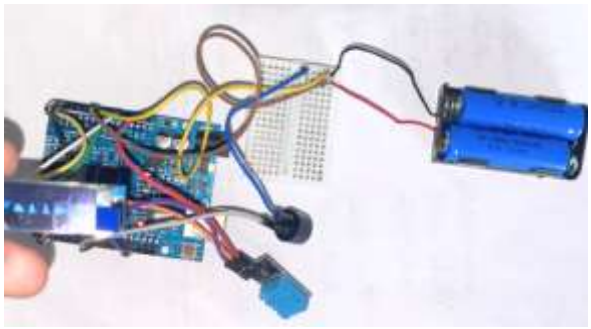


Figure 4. *Final Monitoring Device*

The DHT11 senses the numerical data of the temperature and humidity of its surroundings. The data is then transferred and shown on the SSD1306 128x32 display. The buzzer goes off when the DHT11 detects that the temperature and humidity exceed the given thresholds. It acts as an alarm, indicating to the farmer to take a specific action to avoid the threat of fungi or confirm its presence, mainly through the ALERT Kit.

Application of Real-Time Monitoring System

To ensure the proper application of the real-time monitoring device, the cacao samples undergoing fermentation were divided into three variables: S1 (infected), S2 (healthy), and S3 (natural), wherein only S3 was monitored continuously until the buzzer made a loud noise, informing the researchers of the possible presence of fungi.

Application of the Kit

Triage Screening

The samples were soaked three times in different solutions: diluted bleach, distilled water, and another distilled water. The sample stood for 10 minutes in the third solution (distilled water). Then, the five (5) sample drops were transferred to a microcentrifuge tube. Five (5) drops of magnetic nanoparticles were added. Then, the sample was put in the magnetic rack. The sample stayed standing for 5 minutes. A positive result for A. *flavus* will result in wide scattering. Meanwhile, negative results will manifest as clumped magnetic particles.

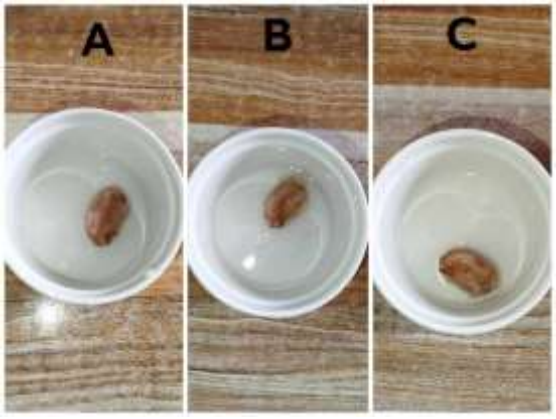


Figure 5: Soaking the sample in bleach (A), Rinsing in distilled water (B), Soaking in distilled water (C).

DNA Extraction

Five (5) drops of solution A (PBS Solution) were added to the sample. Then, 10 drops of solution B (Lysis Buffer) were added to the sample. The sample stayed standing for 5 minutes. After, a dipstick was put in the MNP-Lysis sample for 10 seconds. Then, solution C or a wash buffer was prepared in a new microcentrifuge tube. The dipstick was soaked in the new tube. Then, the dipstick was removed, and one (1) drop of solution D (TE Buffer) was added.

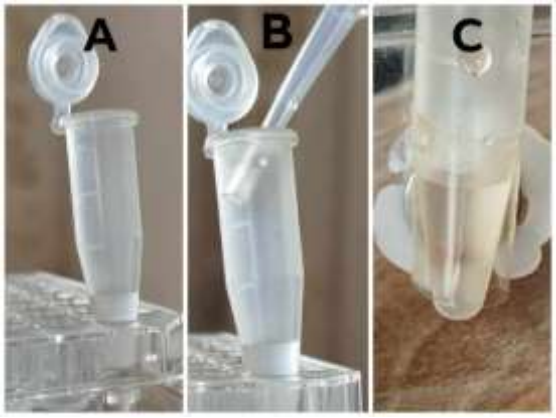


Figure 6: Transfer five (5) drops of the sample to a clean tube (A). Add five (5) drops of MNP (magnetic nanoparticles) (B). Add 3 drops of Solution A (Phosphate Buffer Saline Solution) (C).

Colorimetric Sensing

One (1) drop of all samples was inserted in the respective microcentrifuge, where one drop of AFIR4 was added. One (1) drop of gold nanoparticles was added and set to sit for five (5) minutes at room temperature. After one (1) drop of 0.1 M hydrochloric acid was added and left to rest for another ten (10) minutes. After that, the researchers are to observe the color shown in the centrifuge; if it is positive or shows the presence of aflatoxin, it will stay pink/red; if it is negative, it will be purple or gray.

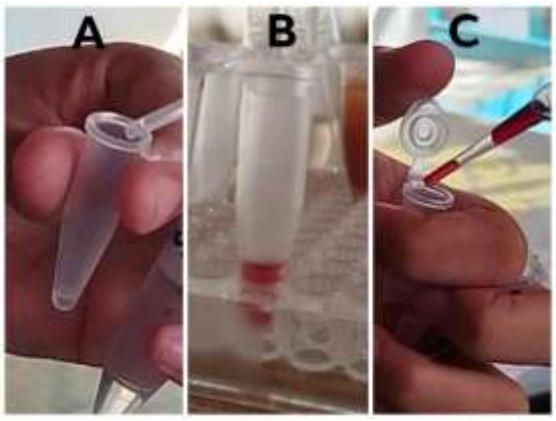


Figure 7: Add one (1) drop of gold nanoparticles (A) Adding hydrochloric acid (B) Results of the colorimetric (C).

Data Collection

Environmental temperature and relative humidity were recorded at 09:00 AM, 11:00 AM, 2:00 PM, 4:00 PM, 7:00 PM, and 9:00 PM. These initial measurements provided the foundation for the subsequent analysis using the pathogen test kit to detect the potential presence of fungi.

The ALERT test kit employed a colorimetric sensing approach. A darker pigment indicated a positive result for fungal presence, while a lighter pigment or the absence of pigment signified a negative result.

Compliance of PCR Test for Reference Standard

One sample of each variable was sent to the Philippine Genome Center—Mindanao (PGCM) for analysis. PGC Mindanao, located at UP Mindanao's College of Science and Mathematics building, is a satellite facility of the Philippine Genome Center (PGC) and aims to utilize omics technology for scientific development in Mindanao. Samples were sent on the same day as the ALERT Kit application to guarantee reliable results. The laboratory results from PGC Mindanao will validate and confirm the findings obtained using the ALERT Kit.

Data Analysis

The researchers utilized qualitative data analysis to gain a detailed understanding of the experiment's findings beyond just the numbers. This involves collecting non-numerical data, such as interviews and surveys, to understand participant experiences and perspectives. By studying this information, the researchers aim to uncover the reasons behind the results and identify unexpected patterns. Qualitative analysis adds meaning and context to the experiment's outcomes (Lim, 2024).

Furthermore, the researchers employed Qualitative Comparative Analysis (QCA), a research method combining qualitative and quantitative techniques to understand how various factors relate to a specific outcome. Instead of looking at individual variables in isolation, QCA analyzes cases as combinations of conditions. This method is beneficial for studies with a medium number of cases, where traditional qualitative or statistical methods might not be ideal. QCA helps researchers understand that different paths can lead to the same result (Thomann et al., 2022).

Waste Disposal

Contaminated wastes were disposed of carefully and safely to prevent microbial growth. The plant pathogen test generated biological waste, including used test kits, plant samples, and pathogen-contaminated materials. Researchers wore personal protective equipment (Battista et al., 2021) when handling the contaminated waste. This waste was disposed of in a separate container, tightly sealed to prevent leaks or contamination (Gerba, 2020). Chemical wastes, such as reagents and buffers, were disposed of in a biohazard container. Finally, the researchers practiced disinfection and sterilization to eradicate infectious microbes (McDonnell, 2020).

Risk and Safety

Adherence to the provided steps and guidelines was mandatory for correctly utilizing the plant pathogen test kit. Engaging with microbial organisms presented potential dangers. Because the researchers were dealing with *A. flavus* and potentially other plant pathogens, the test was performed cautiously and attentively to protect them from potential hazards. The researchers wore protective equipment (PPE) when handling the plant pathogen, per laboratory guidelines. Extensive care was taken to ensure safety when handling microorganisms during the experimental procedure (World Health Organization, 2024).

Research Design

The researchers utilized two research designs. The first phase of the study is experimental design, in which the researchers conducted scientific experiments and trials in the programming of the algorithm to prove their hypothesis that an Arduino-based device can keep track of the current state of the environment and monitor the acquired data, which are the thriving factors of A. *flavus*, mainly the environmental temperature and relative humidity.

The study's second phase included a validation design, which evaluated the functionality of the ALERT Plant Pathogen Test Kit and compared it to a standard pathogen detection method, the polymerase chain reaction (PCR) test.

Scope and Limitation

The study assessed the early detection of fungi, specifically A. *flavus*, using the monitoring device and the ALERT Kit, which used the three variables (healthy, infected, and natural). Limitations are the exclusion of specific identification of the A. *flavus* species by doing the highly sensitive PCR testing or the capillary PCR testing together due to the lack of an isolated culture of A. *flavus.*

RESULTS

The environmental temperature and relative humidity of S3 were monitored throughout the day, namely at 9:00 AM, 11:00 AM, 2:00 PM, 4:00 PM, 7:00 PM, and 9:00 PM, until it reached the threshold level and the buzzer made a loud noise. To confirm the presence of A. *flavus*, the ALERT Kit was used on the cacao beans. Results collected from the ALERT Kit were then qualitatively compared, and for further validation, they underwent PCR testing.

Monitored Temperature and Humidity

Table 2 shows the temperature and humidity monitored by the device throughout three (3) straight days. The device detected the temperature and humidity for

Table 2: Temperature and humidity monitored by the monitoring device (value in **BOLD** is the time were the conditions reached the threshold).

	DAY 1	DAY2	DAY3
9:00 a.m	31.8°C, 98%	29.8°C 98%	33.3°C, 98%
11:00 a.m	31.3°C, 98%	31.8°C, 98%	32.3°C, 98%
2:00 p.m	32.3°C, 98%	33.3°C, 98%	**37.4°C, 98%**
4:00 p.m	31.3°C, 98%	33.3°C, 98%	36.5°C, 98%
7:00 p.m	30.8°C, 98%	32.8°C, 98%	33.3°C, 98%
9:00 p.m	30.2°C, 98%	32.8°C, 98%	31.2°C, 98%

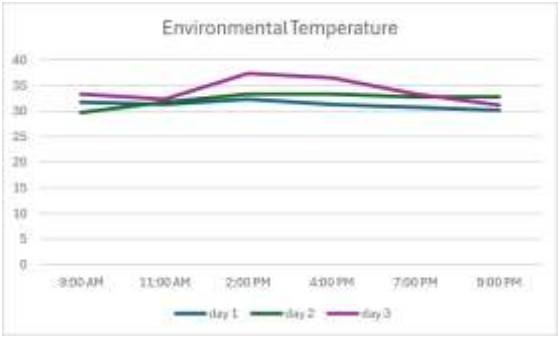


Figure 8: Line graph of the temperature monitored by the device for consecutive three (3) days.

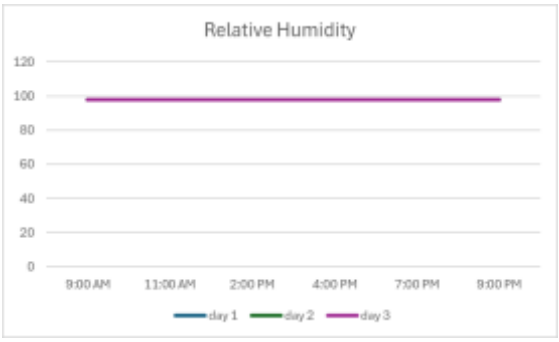
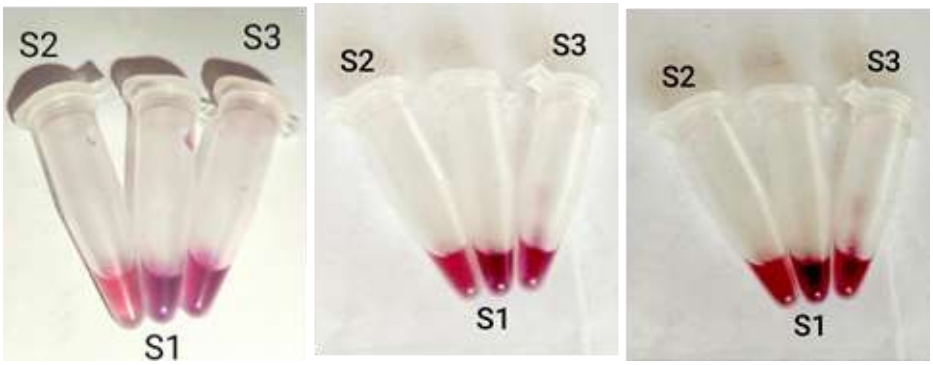


Figure 9: Line graph of the relative humidity monitored by the device for consecutive three (3) days.

Results from the Application of the ALERT Kit



Trial 1 Trial 2 Trial 3

Figure 10: *Colorimetric Results. Positive + darker pigment, negative = lighter to no pigment.*

Results from the PCR testing

Table 3: *Agarose Gel Electrophoresis Results.*

PGC ID	Result	Rating
S1	**Positive**	**Passed**
S2	**Negative**	**Passed**
S3	**Positive**	**Passed**

The table shows the results of the PCR testing done by the PGCM. The results showed that the S2 (healthy samples) showed no sign of fungi infection. Meanwhile, the S1 and S3 (intentionally and naturally infected) showed that fungi were present in their DNA extract.

DISCUSSIONS

The average temperature and humidity in Table 1 showed accurate conditions in which fungi might be present. The initial detection of *Aspergillus flavus* in cacao bean samples using a monitoring system device was a crucial first step in assessing potential contamination, with subsequent validation achieved through the ALERT Test kit. The congruence between the device readings and the ALERT Test kit results was further corroborated by laboratory-based polymerase chain reaction (PCR) testing, enhancing the reliability of the detection process (Franco et al., 2019). This multi-faceted validation approach underscores the potential of the monitoring system device and the ALERT Test kit as valuable tools for preliminary screening and assessment of A. *flavus* presence in cacao beans.

The observed environmental temperature and relative humidity trends demonstrate an apparent diurnal variation. Measurements taken at 09:00 AM, 11:00 AM, and 2:00 AM hours showed a progressive increase, while values at 4:00 PM, 7:00 PM, and 9:00 PM exhibited a corresponding decrease. Consequently, we infer that maximum temperatures are typically reached between late morning and early afternoon, with temperatures declining

throughout the late afternoon and evening (Chinazzo et al., 2022). Furthermore, we infer that fermented cacao beans exhibit heightened susceptibility to fungal infection from 09:00 AM to 2:00 PM, potentially due to the prevailing environmental conditions of increasing temperature observed during this time (Subroto et al., 2023).

The experiment/observation period was characterized by a consistently high relative humidity of 98%, suggesting a highly saturated environment. The enclosed nature of the experimental setup may have contributed to this stable, high humidity, effectively fixing it (*Relative Humidity and Temperature*, n.d.-b). To improve the robustness of future investigations, the influence of this consistently high humidity should be carefully considered and controlled.

The observed agreement between visual assessments of cacao bean samples (categorized as infected, healthy, and naturally infected/healthy) and PCR outcomes carries substantial significance. It not only validates the accuracy of visual inspection for rapid, on-site evaluations but also reinforces the reliability of the PCR assay in identifying pathogens or health indicators. This alignment is particularly beneficial in classifying ambiguous samples, thereby improving our understanding of early or latent infections and supporting the use of visual assessment as a complementary method to laboratory techniques.

The consistency across the monitoring system device, ALERT Test kit, and PCR results suggest the potential utility of the ALERT device as a monitoring system during cacao bean fermentation to detect factors exacerbating fungal growth. Furthermore, compared to laboratory findings, the ALERT Kit's accurate and consistent results indicate its potential as a cost-effective, rapid, and user-friendly method for pathogen detection. While these findings are promising, further comprehensive studies are warranted to validate these results across diverse cacao varieties, geographical locations, and fermentation conditions, ensuring broad applicability and robustness of the proposed monitoring and detection methods.

CONCLUSION

This study aimed to verify the effectiveness of the ALERT Kit as a tool to confirm the suspected A and to use a device to perform a real-time monitoring system for the fermenting cacao beans *A. flavus* that the device initially suspected.

The device was functional in monitoring the real-time environmental temperature and relative humidity. The beans detected early as infected were tested with the ALERT Kit and tested positive for fungal infection. The samples were sent to a molecular biology laboratory for further testing for polymerase chain reaction (PCR) tests. The laboratory results manifested as matching the previous results (positive). The test was also matched to the other two variables (intentionally healthy bean and intentionally infected bean).

These results indicate the device's potential for real-time monitoring of cacao beans in plantations, enabling early detection of fungal infections. These results indicate the device's potential for real-time monitoring of cacao beans in plantations, enabling early detection of fungal infections. However, additional studies will be undertaken to validate this research across diverse conditions comprehensively.

RECOMMENDATIONS

For future research, it is recommended to focus on three key areas: conducting the sensor's capability in an on-field setting, sending more samples to be tested, and expanding the number of sensors for detection. More samples to confirm the presence of fungi in the beans would help prove the validity of the ALERT Kit, while field testing will confirm the device's efficacy in real-time monitoring. Furthermore, incorporating an additional sensor into the device can provide further foundation for early cocoa detection.

ACKNOWLEDGEMENT

Without the blessings and direction of the Almighty God, to whom we are thankful for his presence during the experimentation process, this research would not have been feasible. His leadership and generosity are responsible for the research's conclusion.

Additionally, we would like to express our appreciation to our parents and other family members who have helped us along the way; we sincerely appreciate their efforts and encouragement. We also deeply appreciate our classmates, who have advised us and suggested many sites for finding qualified scientists, suitable labs to contact, and other networks to connect with.

Our most profound appreciation is sent to our respected research mentor, Felix Robert Valenzuela, and our research III & IV teacher, Chito Napitan, EdD. Their ideas, mentorship, and overall support guided us throughout the conducting of the research. Their unwavering support has been a key to our successful research.

Appreciation is extended to the Department of Science and Technology - Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (DOST-PCAARRD) for funding the research. This opportunity served as the key to our idea and the success that we achieved in our study.

CONFLICT OF INTEREST

Each author declares no conflict of interest.

REFERENCES

Abdurasulova, D. (2023). C++ Programming Language FOR IMPLEMENTATION OF SORTING ALGORITHMS. *Journal of technical research and development*, *1(2).*

Al-Zaban, M. I. (2023). Impacts of Temperature and Water Activity Interactions on Growth, Aflatoxin B1 Production and Expression of Major Biosynthetic Genes of AFB1 in Aspergillus flavus Isolates. *Microorganisms*, *11*(5), 1199

Artika, I. M., & Ma'roef, C. N. (2017). Laboratory biosafety for handling emerging viruses. *Asian Pacific journal of tropical biomedicine*, *7*(5), 483-491.

Battista, R. A., Ferraro, M., Piccioni, L. O., Malzanni, G. E., & Bussi, M. (2021). Personal protective equipment (PPE) in COVID 19 pandemic: related symptoms and adverse reactions in healthcare workers and general *population. Journal of occupational and environmental medicine*, *63*(2), e80-e85.

Chinazzo, G., Andersen, R. K., Azar, E., Barthelmes, V. M., Becchio, C., Belussi, L., ... & Wei, S. (2022). Quality criteria for multi-domain studies in the indoor environment: Critical review towards research guidelines and recommendations. *Building and Environment*, *226*, 109719.

Enespa, N., & Chandra, P. (2021). Aflatoxins: Food safety, human health hazards and their prevention. In *IntechOpen eBooks*. https://doi.org/10.5772/intechopen.96647

Franco, A. J. D., Merca, F. E., Rodriguez, M. S., Balidion, J. F., Migo, V. P., Amalin, D. M., ... & Fernando, L. M. (2019). DNA-based electrochemical nanobiosensor for the detection of Phytophthora palmivora (Butler) Butler, causing black pod rot in cacao (Theobroma cacao L.) pods. *Physiological and Molecular Plant Pathology*, *107*, 14-20.

FAO-WHO Regional Conference on Food Safety for Asia and the Pacific. (n.d.). https://www.fao.org/4/AD729E/AD729E00.htm?fbclid=IwZXh0bgNhZW0CMTEAAR1x0ejqYv-f_Ryby9eMF_0urfiWazD-NQ_sGV83xmHT1JpLAvE-Ww0iUqQ_aem_R7UMIDFR9iQ0W49CKdnd-A

Fernando-Corpuz, L. (2024). DNA-Based Nanobiosensor Detection [Brochure] University of the Philippines, Los Baños, Laguna.

Gerba, C. P. (2020). Microbial pathogens in municipal solid waste. *In Microbiology of solid waste* (pp. 155-174). CRC Press.

Jaibangyang, S., Nasanit, R., & Limtong, S. (2021). Effects of temperature and relative humidity on Aflatoxin B1 reduction in corn grains and antagonistic activities against Aflatoxin-producing Aspergillus flavus by a volatile organic compound-producing yeast, Kwoniella heveanensis DMKU-CE82. *BioControl*, *66*(3), 433-443.

Lim, W. M. (2024). What is qualitative research? An overview and guidelines. *Australasian Marketing Journal*, 14413582241264619.

Medenilla, V. (2022). Towards greener pastures: cacao farming gave a farmer sustainable means to support the needs of her children. *Agriculture (Philippines)*, *26*(9).

McDonnell, G. E. (2020). *Antisepsis, disinfection, and sterilization: types, action, and resistance.* John Wiley & Sons.

Naresh, V., & Lee, N. (2021). A review on biosensors and recent development of nanostructured materials-enabled biosensors. Sensors, 21(4), 1109.

Pangga, I. B., Salvacion, A. R., & Cumagun, C. J. R. (2015). Climate change and plant diseases caused by mycotoxigenic fungi: Implications for food security. *Climate change and mycotoxins*, 1-28.

Pfliegler, W. P., Pócsi, I., Győri, Z., & Pusztahelyi, T. (2020). The Aspergilli and Their Mycotoxins: Metabolic Interactions With Plants and the Soil Biota. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.02921

Prasetia, H. A., Panjaitan, L., Susilo, B. H., Budiawan, S., Suherman, B., Handayani, N. D., ... & Dikin, A. (2024). The effects of various storing temperatures in suppressing Aspergillus flavus and mycotoxin contaminations in hermetically packed dried nutmeg (Myristica fragrans Houtt) seed. *Journal of Stored Products Research*, *109*, 102403.

Relative humidity and temperature. (n.d.-b). Retrieved February 25, 2025, from https://manual.museum.wa.gov.au/book/export/html/89/index.html

Subroto, E., Djali, M., Indiarto, R., Lembong, E., & Baiti, N. (2023). Microbiological activity affects post-harvest quality of cocoa (Theobroma cacao L.) beans. *Horticulturae*, *9*(7), 805.

Tai, B., Chang, J., Liu, Y., & Xing, F. (2020). Recent progress of the effect of environmental factors on Aspergillus flavus growth and aflatoxins production on foods. *Food Quality and Safety*, *4*(1), 21-28.

Thomann, E., Ege, J., & Paustyan, E. (2022). Approaches to Qualitative Comparative Analysis and good practices: *A systematic review. Swiss political science review*, *28*(3), 557-580.

World Health Organization. (2024). Laboratory biosecurity guidance. World Health Organization

Appendix (A)

Republic of the Philippines
City Agriculturist's Office
City of Davao

CERTIFICATION

This is to certify that **MIKAELLAH R. PAMA and others** from Daniel R. Aguinaldo National School High School, located at Matina, Davao City, have submitted specimen of **Cacao *(Theobroma cacao L.)*** pods to this office for authentication and verification.

We further certify that the submitted specimen is true to type for **Cacao *(Theobroma cacao L.)*** pods.

This certification is issued for any legal purpose that may be required.

Done this **1st** day of **October**, **2024** in Davao City.

DR. ANTONIO R. OBSIOMA
Officer-in-Charge

4th Floor, City Hall Annex Bldg., A. Pichon Sr. St., Davao City
228-6582 / 241-1000 loc. 359-363 agri@davaocity.gov.ph

Appendix (B)

COLLEGE OF AGRICULTURE AND FOOD SCIENCE
UNIVERSITY OF THE PHILIPPINES LOS BAÑOS

Nemesio B. Mendiola Hall, UP Los Baños, College, Laguna, 4031
+63 49 501 6649| icrops.uplb@up.edu.ph

INSTITUTE OF CROP SCIENCE

01 October 2024

CERTIFICATION

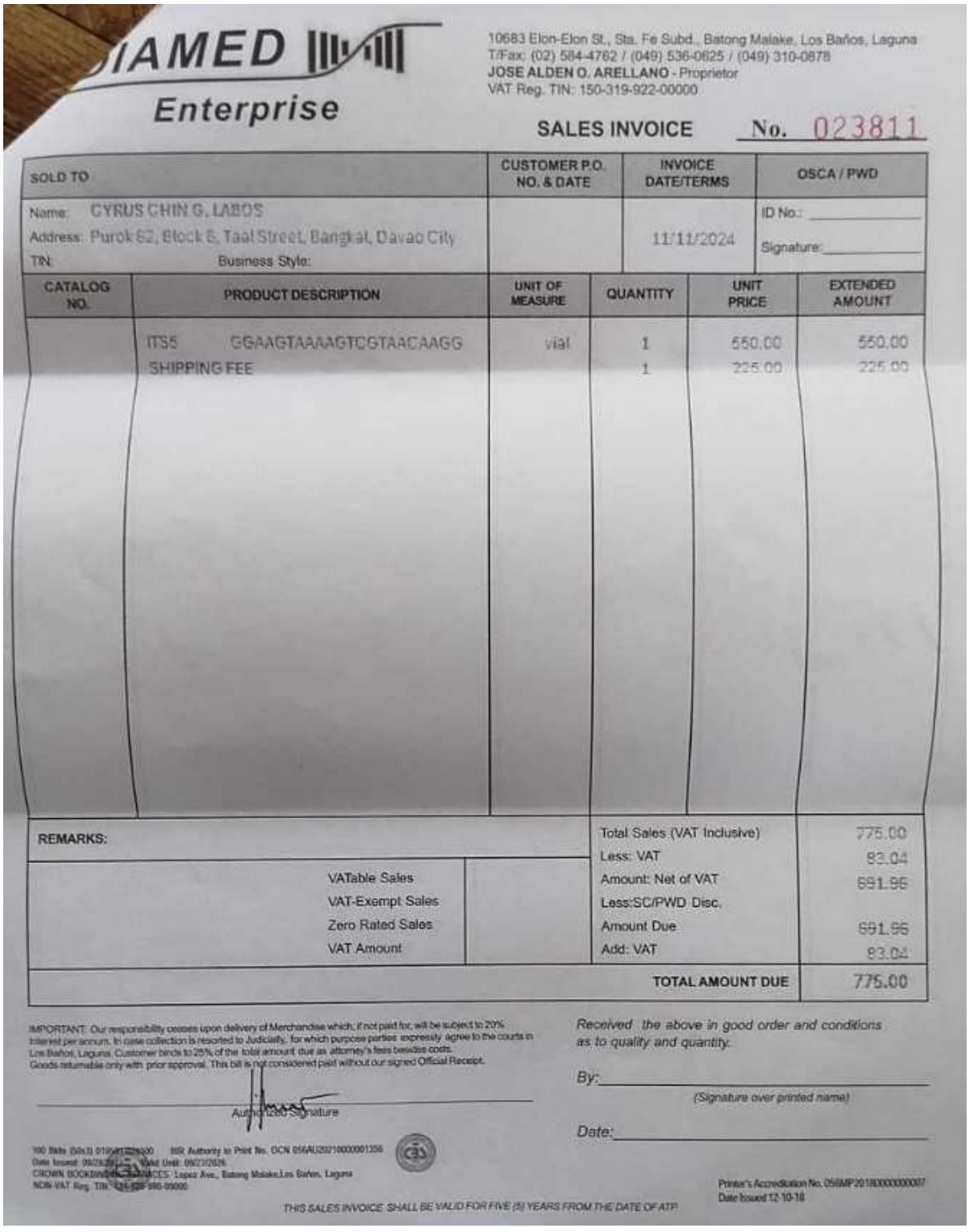
This is to certify that the oligonucleotide probes for *Aspergillus* spp. (*Aspergillus flavus* and *Aspergillus oryzae*) provided by my laboratory to April Rose Melana, Cyrus Chin Labos, and Mikaellah Pama of the Daniel R. Aguinaldo National High School (DRANHS) to be used for the Division's Science and Technology Fair (DSTF) was designed specific to the abovementioned microorganism through the UPLB-DOST-PCAARRD project entitled **"Quick Detection of Mycotoxins in Cocoa using Nanobiosensor"**.

Thank you.

Very truly yours,

LILIA M. FERNANDO-CORPUZ, PhD
Associate Professor and Project Leader

Appendix (C)



DIAMED Enterprise

10683 Elon-Elon St., Sta. Fe Subd., Batong Malake, Los Baños, Laguna
T/Fax: (02) 584-4762 / (049) 536-0625 / (049) 310-0878
JOSE ALDEN O. ARELLANO - Proprietor
VAT Reg. TIN: 150-319-922-00000

SALES INVOICE No. 023811

SOLD TO	CUSTOMER P.O. NO. & DATE	INVOICE DATE/TERMS	OSCA / PWD
Name: CYRUS CHIN G. LABOS Address: Purok 62, Block 6, Taal Street, Bangkal, Davao City TIN: Business Style:		11/11/2024	ID No.: _____ Signature: _____

CATALOG NO.	PRODUCT DESCRIPTION	UNIT OF MEASURE	QUANTITY	UNIT PRICE	EXTENDED AMOUNT
	ITS5 GGAAGTAAAAGTCGTAACAAGG	vial	1	550.00	550.00
	SHIPPING FEE		1	225.00	225.00

REMARKS:			
		Total Sales (VAT Inclusive)	775.00
		Less: VAT	83.04
VATable Sales		Amount: Net of VAT	691.96
VAT-Exempt Sales		Less:SC/PWD Disc.	
Zero Rated Sales		Amount Due	691.96
VAT Amount		Add: VAT	83.04
		TOTAL AMOUNT DUE	775.00

IMPORTANT: Our responsibility ceases upon delivery of Merchandise which, if not paid for, will be subject to 20% interest per annum. In case collection is resorted to Judicially, for which purpose parties expressly agree to the courts in Los Baños, Laguna. Customer binds to 25% of the total amount due as attorney's fees besides costs.
Goods returnable only with prior approval. This bill is not considered paid without our signed Official Receipt.

Authorized Signature

Received the above in good order and conditions as to quality and quantity.

By: _____
(Signature over printed name)

Date: _____

100 Bills (50x3) 019[illegible]00 BIR Authority to Print No. OCN 056AU20210000001356
Date Issued: 09/28/[illegible] Valid Until: 09/27/2026
CROWN BOOKBINDING SERVICES Lopez Ave., Batong Malake,Los Baños, Laguna
NON-VAT Reg. TIN: [illegible]

Printer's Accreditation No. 056MP20180000000007
Date Issued 12-10-18

THIS SALES INVOICE SHALL BE VALID FOR FIVE (5) YEARS FROM THE DATE OF ATP

Appendix (D)

```
#include <Wire.h>
#include <Adafruit_GFX.h>
#include <Adafruit_SSD1306.h>
#include <DHT.h>

#define SCREEN_WIDTH 128
#define SCREEN_HEIGHT 64
#define DHTPIN 2
#define DHTTYPE DHT11
int BUZZER_PIN = 8;

Adafruit_SSD1306 oled(SCREEN_WIDTH, SCREEN_HEIGHT, &Wire, -1);
DHT dht(DHTPIN, DHTTYPE);

String temperature;
String humidity;

void oledDisplayCenter(String temperature, String humidity) {
  int16_t x1, y1;
  uint16_t width_T, height_T, width_H, height_H;

  oled.getTextBounds(temperature, 0, 0, &x1, &y1, &width_T, &height_T);
  oled.getTextBounds(humidity, 0, 0, &x1, &y1, &width_H, &height_H);


  oled.clearDisplay();
  oled.setCursor((SCREEN_WIDTH - width_T) / 2, SCREEN_HEIGHT / 2 - height_T - 5);
  oled.println(temperature);
  oled.setCursor((SCREEN_WIDTH - width_H) / 2, SCREEN_HEIGHT / 2 + 5);
  oled.println(humidity);
  oled.display();
}

void setup() {
  Serial.begin(9600);
 pinMode(BUZZER_PIN, OUTPUT);

  // Initialize OLED display
  if (!oled.begin(SSD1306_SWITCHCAPVCC, 0x3C)) {
    Serial.println(F("SSD1306 allocation failed"));
    while (true);
  }

  delay(2000);
  oled.clearDisplay();

  oled.setTextSize(3);      // Set text size
  oled.setTextColor(WHITE);  // Set text color
  oled.setCursor(0, 10);     // Set cursor position

  // Initialize DHT11 sensor
  dht.begin();

  temperature.reserve(10);
  humidity.reserve(10);

}

void loop() {
  // Read temperature and humidity from DHT11
  float humi = dht.readHumidity();
  float tempC = dht.readTemperature();

  // Check if readings are valid
  if (isnan(humi) || isnan(tempC)) {
    temperature = "Failed";
    humidity = "Failed";
  } else {
    // Format temperature and humidity
    temperature = String(tempC, 1) + char(247) + "C"; // 247 is the degree symbol
    humidity = String(humi, 1) + "%";
  }

  // serial monitor display
  Serial.print("Temperature: ");
  Serial.print(tempC);
  Serial.print("°C | Humidity: ");
  Serial.print(humi);
  Serial.println("%");

  // OLED display
  oledDisplayCenter(temperature, humidity);

  if (tempC > 29.0 && humi > 80.0) {
    digitalWrite(BUZZER_PIN,HIGH); // Turn buzzer on
  } else {
    digitalWrite(BUZZER_PIN,LOW); // Turn buzzer off
  }

  delay(2000);
}
```